GLEANINGS FROM MY RESEARCHES
Vol. II
Malaria, Hæmolysis and other Subjects

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MALARIA, HÆMOLYSIS AND OTHER SUBJECTS

BY

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The *gleaners* spread around, and here and there,
Spike after spike, their scanty harvest pick.
                    Thomson. Autumn, 1.165.

DEDICATED TO MY FAITHFUL ASSISTANTS AND STUDENTS
LIVING OR DEAD WHO UNGRUDGINGLY HELPED ME
IN MANY DIFFICULT AND DISTRESSING
DAYS OF MY RESEARCH

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FOREWORD

The second volume of my *Gleanings from my Researches* consists mostly of my work on malaria and hæmolysis. The first chapter contains that portion of my address read before the Indian Science Congress, 1938, which relates to *Certain Observations on the Chemotherapy of Malaria*. The volume also contains a few chapters on kala-azar which could not be incorporated in the first volume. There is a reference to an article of Sir Leonard Rogers entitled *Antimony Treatment of Kala-azar* which was published in *Nature*, December, 1939, in which he has not done justice to the pioneers in the antimony treatment of the disease.

In this work are also incorporated my researches in black-water fever, anophelines, chemistry and chemotherapy of quinoline compounds and therapeutic properties of a compound allied to atebrin named by me acridin X.

It also contains my papers in other subjects and in clinical medicine consisting of reports of interesting cases that were published from time to time in my younger days including the first series of the recorded cases of quartan fever in India.

I am indebted to Rai Bahadur Dr. K. N. Bagchi, M.B., B.Sc., D.T.M., F.I.C., F.N.I., Chemical Examiner to the Government of Bengal, for correcting the proofs of my papers. Without his aid I would not have been able to complete my book.

LIST OF ILLUSTRATIONS*

* I am indebted to various journals for articles and diagrams reproduced in this book.

CERTAIN OBSERVATIONS ON THE CHEMOTHERAPY OF MALARIA

[This is the second part of the author's Presidential Address, Section of Medical Research, Twenty-fifth Session of the Indian Science Congress, Calcutta, 1938. The first portion under the heading of *The Conquest of Kala-azar* is published in the first volume.]

I now pass on from the conquest of kala-azar, to certain observations on the chemotherapy of antimalarial quinoline and acridine compounds, which may one day play an important part in the campaign against malaria and its conquest.

In most of the eastern parts of India malaria and kala-azar go together. They have many symptoms in common and were confused with each other for a long time.

Research in chemotherapy of malaria has been intense in recent times. In certain parts of India it is of greater interest than kala-azar. For sometime past, work in this line has been undertaken by the speaker and co-workers and new quinoline compounds synthesized with the view of studying their action on paramœcia as well as on the parasites of malaria. While, as will be seen from the accompanying tables, some of these compounds have marked destructive action on paramœcia, most of them have been observed to have no action on the parasites of malaria when used clinically in patients suffering from the disease.

TABLE I

Action of certain quinoline compounds on paramœcia

	STRENGTH	EFFECT ON PARAMŒCIA
I. 6-amino-quinoline NH_2	1 : 2,000 1 : 4,000	Death No death
II. Quinoline-6-glycine-amide $NH_2 \cdot CO \cdot CH_2 \cdot NH$	1 : 2,000 1 : 4,000	Death No death
III. 8-amino-quinoline NH_2 N	1 : 2,000 1 : 4,000	No death No death
IV. Quinoline-8-glycine-amide $NH_2 \cdot CO \cdot CH_2 \cdot NH$ N	1 : 2,000	No death
V. 6-oxy-8-amino-quinoline HO NH_2 N	1 : 2,000 1 : 10,000 1 : 20,000 1 : 40,000 1 : 80,000 1 : 160,000 1 : 320,000	Death in 6 minutes Death in 7 minutes Death in 7 minutes Death in 8 minutes Death in 14 minutes Death in 17 minutes No death in 1 hour
VI. 6-oxy-quinoline-8-glycine-amide HO $NH_2 \cdot CO \cdot CH_2 \cdot NH$ N	1 : 200 1 : 10,000 1 : 20,000 1 : 40,000 1 : 80,000 1 : 160,000 1 : 320,000	Death in 7 minutes Death in 7 minutes Death in 10 minutes Death in 11 minutes Death in 15 minutes Death in 19 minutes No death
VII. 6-methoxy-quinoline-8-glycine-amide CH_3O $NH_2 \cdot CO \cdot CH_2 \cdot NH$ N	1 : 1,000	No action

For purpose of comparison we append here the action of quinine hydrochloride on paramœcia under conditions similar to the above :

	STRENGTH	EFFECT ON PARAMŒCIA
Quinine hydrochloride	1 : 10,000 1 : 20,000 1 : 40,000 1 : 80,000 1 : 160,000	Death in 5 minutes Death in 10 minutes Death in 19 minutes Death in 35 minutes No death in 1 hour

TABLE II

Action of certain quinoline compounds on paramœcia

	STRENGTH	EFFECT ON PARAMŒCIA
I. 6-amino-4-phenyl-quinaldine hydrochloride	1 : 2,000	No death in 1 hour
	1 : 10,000	No death in 1 hour
II. 8-amino-4-phenyl-quinaldine hydrochloride	1 : 2,000	Death in 4 minutes
	1 : 10,000	Death in 24 minutes
	1 : 20,000	Few died in 1 hour
	1 : 40,000	No death in 1 hour

(Reproduced from a paper by the author and co-workers published in the *Journal of Pharmacology and Experimental Therapeutics*, Vol. XLI, No. 3, March, 1931.)

TABLE III

Action of certain quinoline compounds on paramœcia

	STRENGTH	EFFECT ON PARAMŒCIA
1. 8-amino-quinoline-para-arsanilate	1 : 2,000	Few deaths in 1 hour
	1 : 10,000	No death in 1 hour
2. 6-amino-quinoline-para-arsanilate	1 : 2,000	No death in 1 hour
	1 : 10,000	No death in 1 hour
3. 8-amino-ethyl-amino-quinoline hydrochloride (Robinson)	1 : 2,000	Death in 3 minutes
	1 : 10,000	Death in 29 minutes
	1 : 20,000	No death in 1 hour
4. 8-amino isopropyl-amino-quinoline hydrochloride	1 : 2,000	Death in 20 minutes
	1 : 10,000	Death in 35 minutes
	1 : 20,000	90 per cent. died in 1 hour
	1 : 40,000	No death in 1 hour
5. 6-methoxy-8-amino-iso propyl-amino-quinoline di-hydrochloride	1 : 2,000	Few deaths in 1 hour
	1 : 10,000	No death in 1 hour
6. 6-chloro-8-amino-isopropyl-amino-quinoline di-hydrochloride	1 : 2,000	Death in 1 minute
	1 : 10,000	Death in 4 minutes
	1 : 20,000	Death in 12 minutes
	1 : 40,000	Death in 30 minutes
	1 : 80,000	No death in 1 hour
	1 : 100,000	No death in 1 hour
7. 6-chloro-2-methyl-8-amino-isopropyl-amino-quinoline di-hydrochloride	1 : 2,000	Death in 30 minutes
	1 : 10,000	No death in 1 hour
8. Amino-acetyl derivative of 8-amino-ethyl-amino quinoline	1 : 2,000	90 per cent. died in 1 hour
	1 : 10,000	No death in 1 hour

(Reproduced from a paper by P. Brahmachari, U. Brahmachari and R. Banerjea published in the *Journal of Pharmacology and Experimental Therapeutics*, Vol. XLIV, No. 4, April, 1932.)*

TABLE IV

Action of certain quinoline compounds on paramœcia

	Strength	Effect on Paramœcia
1. Allyl-8-amino-quinoline hydrochloride	1 : 2,000 1 : 10,000	Death in 5 minutes No death in 1 hour
2. Allyl-thio-carbamino-8-amino-quinoline hydrochloride	1 : 2,000 1 : 10,000	No death in 1 hour No death in 1 hour
3. 6-methoxy-8-β-dimethyl-amino-isopropyl-amino-quinoline di-hydrochloride	1 : 2,000 1 : 10,000	Death in 14 minutes No death in 1 hour
4. 6 methyl-8-β-dimethyl-amino-isopropyl-amino-quinoline di-hydrochloride	1 : 2,000 1 : 10,000	Death in 1 hour No death in 1 hour
5. 2-methyl-6-methoxy-8-β-dimethyl-amino-iso-propyl-amino-quinoline di-hydrochloride	1 : 2,000 1 : 10,000 1 : 20,000	Death in 28 minutes Death in 1 hour No death in 1 hour
6. Lactyl-8-amino-quinoline hydrochloride	1 : 2,000 1 : 10,000 1 : 20,000 1 : 40,000 1 : 80,000	Death in 12 minutes Death in 28 minutes Death in 35 minutes Death in 1 hour No death in 1 hour
7. β-hydroxy-propyl-8-amino-quinoline hydrochloride	1 : 2,000 1 : 10,000	Death in 45 minutes No death in 1 hour
8. 6-ethoxy β-hydroxy-propyl-8-amino-quinoline-hydrochloride	1 : 2,000 1 : 10,000	No death in 1 hour No death in 1 hour
9. 6-ethoxy-lactyl-8-amino-quinoline-hydrochloride	1 : 2,000 1 : 10,000	Death in 18 minutes No death in 1 hour
10. 6-methoxy-8-β-diethyl-amino-isopropyl-amino-quinoline di-hydrochloride	1 : 2,000 1 : 10,000	No action in 1 hour No action in 1 hour
11. 8-(β-piperidino-isopropyl-amino) quinoline di-hydrochloride	1 : 2,000 1 : 10,000 1 : 20,000 1 : 40,000 1 : 80,000 1 : 100,000	Immediate death Death in 2 minutes Death in 10 minutes Death in 11 minutes Death in 15 minutes Death in 35 minutes

Apart from the variability in the value of the hitherto-known quinoline and acridine derivatives in their destructive action on the different types of malarial parasites or on the schizonts and gametocytes of the same parasite or its strains, these compounds sometimes exhibit toxic symptoms of varying intensity and this is sufficient, justification for further research on synthetic antimalarials.

Of late, a compound having the same composition as quinacrine or atebrin has been synthesized in the author's laboratory. As expected, it has got well-marked antimalarial properties as will be presently seen. It is provisionally named Acridin X or Ax. The compound has been used by him as hydrochloride or hydrobromide. The following is the first series of cases treated in the Tropical Diseases Ward of the Carmichael Medical College Hospitals, Calcutta. Its action on paramœcia is shown in the accompanying table. Its action in monkey malaria has been studied by Shortt and Menon.

Action of Acridin X on Paramœcia

Experiment No. 1

	STRENGTH	EFFECT ON PARAMŒCIA
Acridin X	1: 1,000	Death in 2 to 4 minutes
	1: 5,000	Death in 4 to 6 minutes
	1: 10,000	Death in 8 to 10 minutes
	1: 40,000	Death in 12 to 15 minutes
	1: 80,000	Majority died in 45 minutes
	1: 100,000	Majority died in 75 minutes
	1: 120,000	Few died in 90 minutes
	1: 160,000	No death in 2 hours.

Experiment No. II

	STRENGTH	EFFECT ON PARAMŒCIA
Acridin X {	1: 1,000	Death in 3 minutes
	1: 5,000	Death in 3 to 5 minutes
	1: 10,000	Death in 7 to 10 minutes
	1: 40,000	Death in 10 to 14 minutes
	1: 80,000	Majority died in $1\frac{1}{2}$ hours
	1: 100,000	Majority died in $1\frac{3}{4}$ hours
	1: 120,000	Few died in 2 hours
	1: 160,000	No death in $2\frac{1}{4}$ hours

Experiment No. III

	STRENGTH	EFFECT ON PARAMŒCIA
Acridin X {	1: 1,000	Death in 2 to 5 minutes
	1: 5,000	Death in 4 to 6 minutes
	1: 10,000	Death in 6 to 9 minutes
	1: 40,000	Death in 10 to 15 minutes
	1: 80,000	Majority died in 62 minutes
	1: 100,000	Majority died in $1\frac{1}{2}$ hours (few survived)
	1: 120,000	Few died in 2 hours
	1: 160,000	No death in $2\frac{1}{2}$ hours

A Series of Cases of Malarial Fever treated with Acridin X

No I.—Patient K, æt. 25, was admitted on 4-2-37. History of fever for two months. Patient was put on Ax from 5-2-37 in $1\frac{1}{2}$ grs. doses thrice a day. The effect of the treatment is shown in Chart I. On 4-2-37, B. T. tropho-zoites were present in blood and on 7-2-37 no malarial parasites were found. On 4-2-37 spleen was 3″ below the costal margin and on 11-2-37 it was just felt below the costal margin.

107°F.						
106° ,,		Grs T.D.S.	Grs T.D.S.	Grs T.D.S.	Grs T.D.S.	18 Grs.
105° ,,						
104° ,,		1.5	1.5	1.5	1.5	TOTAL
103 ,,						
102° ,,						
101° ,,	B.T Trophozoit HE found					
100° ,,				Nil		
99° ,,		Do	Do	Parasites	Do	
98° ,,						
97° ,,						
Date.	4/2	5/2	6/2	7/2	8/2	9/2
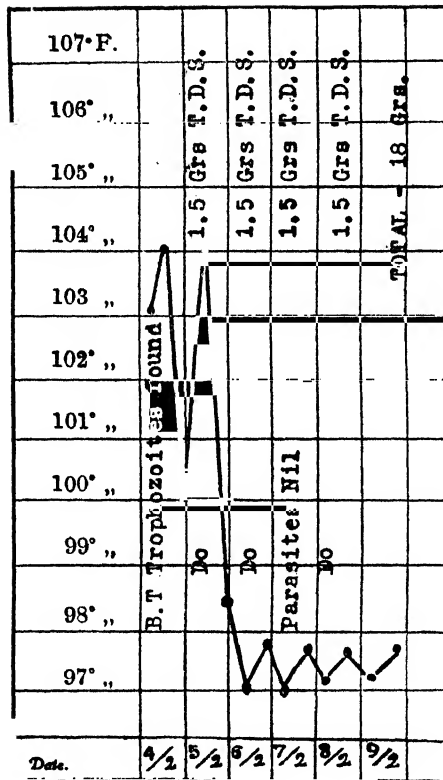
Chart I

Showing the effect of 18 grs. of acridin X (1·5 grs., t.d.s.)
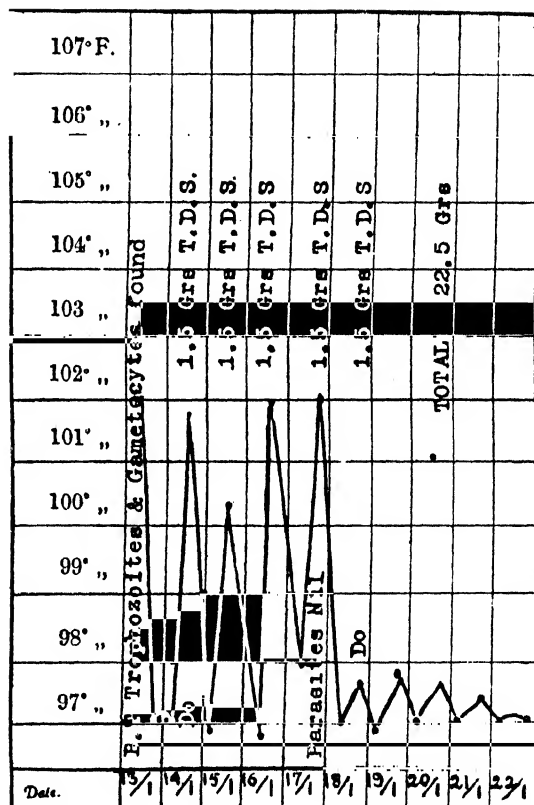
Chart II

Showing the effect of 22·5 grs. of acridin X (1·5 grs., t.d.s.)
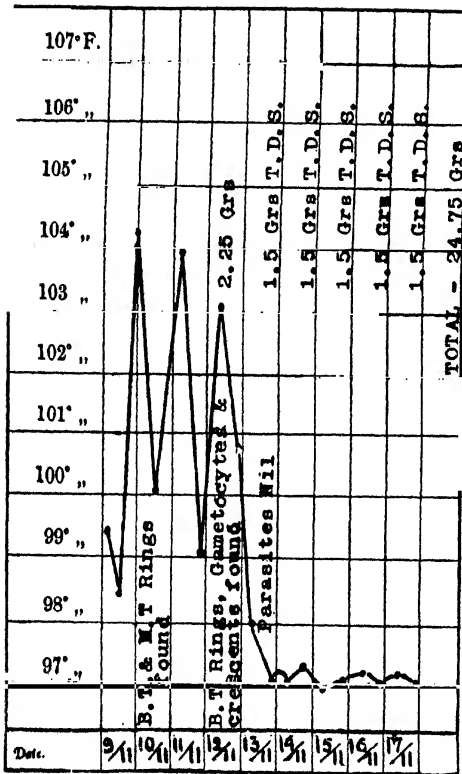
Chart III

Showing the effect of 24·75 grs. of acridin X (initial dose 2·25 grs. and later on 1·5 grs., t.d.s.)
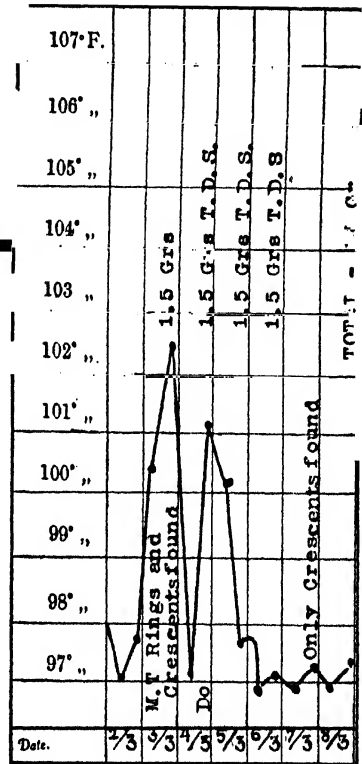
Chart IV

Showing the effect of 15 grs. of acridin X (1·5 grs., t.d.s.)
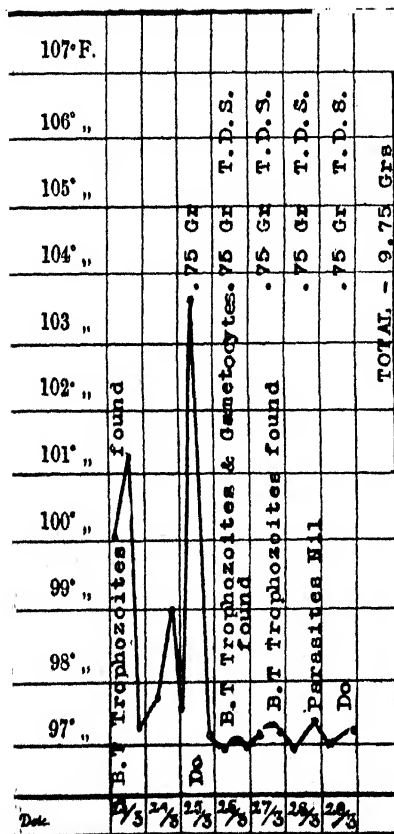
Chart V

Showing the effect of 9·75 grs. of acridin X (0·75 grs., t.d.s.)
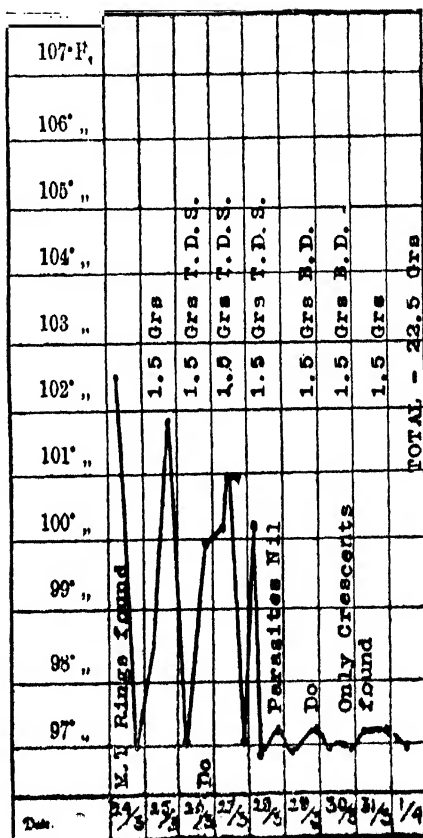
Chart VI

Showing the effect of 22·5 grs. of acridin X (1·5 grs., t.d.s. and later b.d.)

No. II.—Patient B, æt. 20, was admitted on 13-1-37. History of fever for 15 days. Patient was put on Ax from 14-1-37 in $1\frac{1}{2}$ grs. doses thrice a day. The effect of the treatment is shown in Chart II. On 13-1-37 B. T. trophozoites and gametocytes were present in blood and on 17-1-37, no malarial parasites were found. On 13-1-37 spleen was $1\frac{1}{2}''$ below the costal margin and on 8-2-37 it was not palpable at the costal margin.

No. III.—Patient D, æt. 18, was admitted on 9-11-36. History of fever for two months. Patient was put on Ax from 12-11-36 in $2\frac{1}{4}$ grs. doses thrice a day. The effect of the treatment is shown in Chart III. On 10-11-36 B. T. and M. T. trophozoites were present in blood and on 13-11-36 no malarial parasites were found. On 9-11-36 spleen was hard and $2''$ below the costal margin and on 17-2-37 it was just felt at the costal margin.

No. IV.—Patient G, æt. 16, was admitted on 2-3-37. History of fever for $2\frac{1}{2}$ months. Patient was put on Ax from 3-3-37 in $1\frac{1}{2}$ grs. doses thrice a day. The effect of treatment is shown in Chart IV. On 3-3-37 M. T. rings and crescents were present in blood and on 7-3-37 only crescents were found. On 2-3-37 spleen was $1''$ below the costal margin and on 8-4-37 it could just be felt at the costal margin.

No. V.—Patient B, æt. 12, was admitted on 23-3-37. History of fever for 3 days. Patient was put on Ax from 25-3-37 in $\frac{3}{4}$ gr. doses thrice a day. The effect of the treatment is shown in Chart V. On 23-3-37 B. T. trophozoites were present in blood and on 28-3-37 no malarial parasites were found. On 23-3-37 spleen was moderately hard and $2''$ below the costal margin and on 1-4-37 it was just felt at the costal margin.

No. VI.—Patient R, æt. 32, was admitted on 24-3-37. History of fever for 8 days. Patient was put on Ax from 25-3-37 in $1\frac{1}{2}$ grs. doses thrice a day. The effect of treatment is shown in Chart VI. On 24-3-37 M. T. rings

were present in blood and on 30-3-37 few crescents were found. On 24-3-37 spleen was 1½″ below the costal margin and on 9-4-37 it was just felt at the costal margin.

No. VII.—Patient B, æt. 30, was admitted on 13-9-37. History of fever for 28 days. Patient was put on Ax from 16-9-37 in 2¼ grs. doses twice a day. The effect of treatment is shown in Chart VII. On 13-9-37 M.T. trophozoites were present in blood and on 19-9-37 no malarial parasites were found. On 13-9-37 spleen was 1″ below the costal margin and on 27-9-37, it was not palpable.

No. VIII.—Patient R, æt. 30, was admitted on 20-9-37. History of fever for 4 days. Patient was put on Ax from 23-9-37 in 1½ grs. doses twice a day. The effect of treatment is shown in Chart VIII. On 20-9-37 plenty of M.T. rings were present in blood and on 25-9-37 only few crescents were found. On 20-9-37 spleen was 1″ below the costal margin and on 25-9-37 it was not palpable.

No. IX.—Patient M, æt. 15, was admitted on 7-10-37. History of fever for 5 days. Patient was put on Ax from 10-10-37 in 1½ grs. doses twice a day. The effect of treatment is shown in Chart IX. On 8-10-37 B.T. trophozoites were present in blood and on 12-10-37 no malarial parasites were found. On 7-10-37 spleen was 1¼″ below the costal margin and on 15-10-37 it was ½″ below the costal margin.

No X.—Patient G, æt. 20, was admitted on 6-10-37. History of fever for 6 days. Patient was put on Ax from 8-10-37 in 1½ grs. doses twice a day. The effect of the treatment is shown in Chart X. On 6-10-37 M.T. rings were present in blood and on 10-10-37 few crescents were found. On 6-10-37, spleen was 1½″ below the costal margin. On 15-10-37 it was ¼″ below the costal margin.

No.XI.—Patient H, æt. 20, was admitted on 7-10-37. History of fever for 6 days. Patient was put on Ax from 8-10-37 in 1½ grs. doses twice a day. The effect of treatment is shown in Chart XI. On 7-10-37 M.T. rings
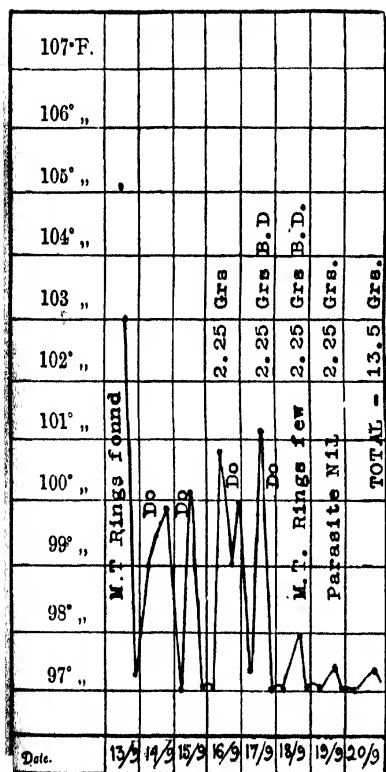
Chart VII

Showing the effect of 13·5 grs. of acridin X
(2·5 grs., b.d.)
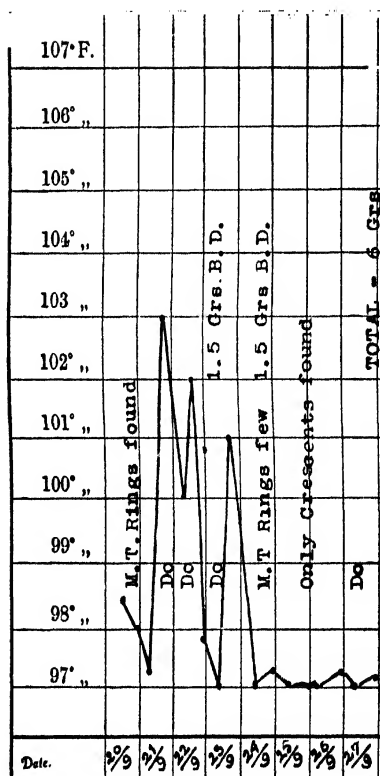
Chart VIII

Showing the effect of 6 grs. of acridin X (1·5 grs.,
b.d)
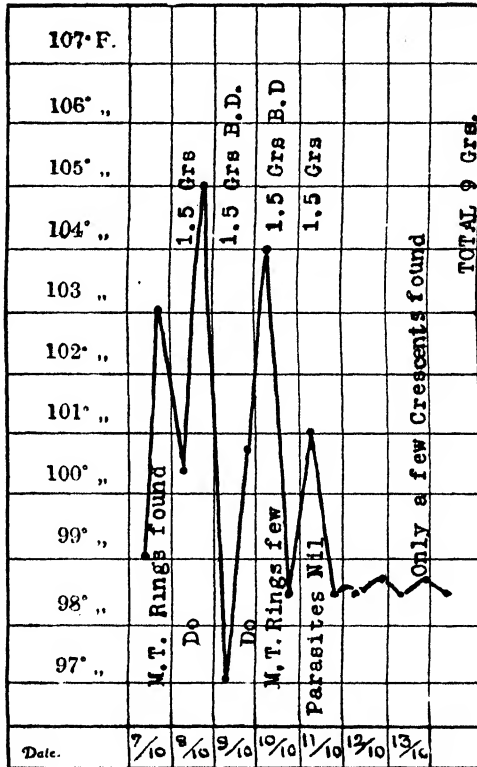
Chart XI

Showing the effect of 9 grs. of acridin X (1·5 grs., b.d.)

were present in blood and on 13-10-37 only a few crescents were found. On 7-10-37 spleen was about 1″ below the costal margin, and on 13-10-37 it was not palpable.

Action of Acridin X in Monkey Malaria.

The action of this drug in monkey malaria has been studied by Shortt and Menon. The following notes are taken from the paper of these authors entitled ' On Acridin X in the Treatment of Monkey Malaria ' (Indian Science Congress Abstracts, Section of Medical Research, 1938) :—

' A series of experiments on Acridin X in the treatment of monkey malaria was carried out on monkeys infected with Plasmodium knowlesi comparing the action of the drug with that of atebrin for injection. The effect in sterilizing the peripheral blood, the cure rate, and relapse rate of the two drugs were compared. The results of the experiments showed the two drugs to be identical in their action on P. knowlesi.'

Up to now the synthetic antimalarials are mostly derived from the quinoline or acridine nucleus. As is well known, it was originally considered that the piperidine nucleus in quinine was responsible for the antimalarial properties of the latter. A few piperidine compounds have been synthesized under the author's direction, but so far they have been found to have no antimalarial properties. It may be stated, en passant, that one of them, namely, piperidino-acetyl-8-amino-6-methoxy-quinoline dihydrochloride, failed to show any antimalarial properties.

The synthesis of organic antimalarials has opened up a new vista in the treatment of malaria and will no doubt play an important part in the future campaign against the disease and its conquest.

The discoveries of effective antimalarials and leishmano-cides rank among the highest triumphs of tropical medicine.

Until recently, they were slumbering, like sleeping beauties, in some unfrequent corners and they now appear to have awakened as fairy gifts which synthetic chemistry bestows from time to time upon mankind.

From what has been stated above, it will be seen that dreadful diseases like malaria and kala-azar will be conquered one day in India and thus a new chapter will be added to ' The Endless Quest.' If along with this conquest there is in India ' a proper balance between labour-saving devices and industry-increasing discoveries,' and if her people ' will but decide to put in play the methods,' which science today has provided for a sufficient supply of food and clothing and shelter, then the health and economic problems of India, with her endless natural resources, will be solved to a great extent ; then much of her unrest and unemployment will cease and she will have the opportunity of being richer, happier, healthier and freer than ever before.

ON THE NATURE OF THE EPIDEMIC FEVER IN LOWER BENGAL COMMONLY KNOWN AS BURDWAN FEVER (1854-75)

There has been a vast literature on the subject. I shall not enter here into a discussion of the origin of the epidemic. The concensus of medical opinion was in favour of the disease being of a malarious nature. There were, however, cases in which the pyrexia was more or less of a continued type. These cases constituted the *Jvar Bikar* of the Ayurvedic practitioners. Some observers thought that these were instances of true typhus (Verchere and Jackson). Jackson subsequently believed that the disease was typhomalarial fever. Greene thought the disease to be typhoid in nature. Lyons thought it was relapsing fever. Roy, French and Wilkie thought that they were cases of ague in which the apyretic intervals had disappeared. Quite recently, Major Rogers has pointed out that the Burdwan fever was precisely similar in nature to the epidemic of kala-azar in Assam. He points out that a few cases terminating rapidly with coma, and doubtless due to cerebral malaria were naturally regarded as part of the epidemic. Lastly, Christophers and Bentley have doubted as to the kala-azar nature of this epidemic.

As the study of the epidemiology of malaria constitutes a part of the investigations with which the provincial malarial

committees are concerned, the following paper is communicated to show that the Burdwan fever was in part, at least, an epidemic malarial fever, *i.e.*, malaria causing, like plague or small-pox or cholera, an epidemic rise in the death curve. I shall not enter in the present paper into the study of the meteorological and physiographical conditions under which the terrible epidemic broke out.

In the *Imperial Gazetteer of India*, Hunter points out that the real Burdwan fever proved fatal within one or two days. In Buckland's *Bengal under the Lieutenant-Governors* the epidemic has been described as a congestive remittent fever running its course to a fatal termination, usually with great rapidity. Such cases were far from uncommon, especially in villages newly affected. Roy states that in such places death from cerebral complications were common, where after a suffering of four or five days, it was not unusual to find people struck down with convulsions, coma and death. According to him, the first attack always proved to be of the continued or remittent type of fever which was looked upon as dangerous. French points out that in one year, 10 per cent of the population of Burdwan had been carried off within two months. In Mahachanda, a village about 7 miles to the north of Burdwan, about one-sixth of the population died in two months (Saunders). Elliot states that the mortality from sudden and great depression of the vital energies was very great in some of the affected places. Gupta wrote that if the first attack of the fever was at all severe and if not checked by prompt and early treatment, it ended in death. The early treatment referred to was treatment by quinine.

In Pandooah where the disease spread in 1862, over 1,200 people died within six months (Elliott). Pillow similarly pointed out that on the first outbreak in a village the disease was of a very rapid and mortal type. The Army Sanitary Commissioner stated in 1872 that the fever was

one of the worst pestilences on record, having in a few months cut off 70 per cent of the population of some of the affected villages. The Inspector-General of Civil Hospitals in his report on the Charitable Dispensaries under the Government of Bengal for the year 1871 stated that the mortality in one outbreak amounted in a few months to one-third the original strength of the community.

The terrible mortality which depopulated Purbusthulee and its adjacent villages in 1862, as mentioned in the petition made by the inhabitants of the place to the Government in the same year, must have been due, to a great extent, to attacks of fever of a similar nature.

I consider that the large majority of cases that died in Oolah or Beernagore must have died of fever of a similar nature. Here, according to Elliott, 10,000 people died in five years' time out of a population of 18,000 ; while, according to other calculations, the mortality amounted to 17,000. It is stated that, at the beginning of the epidemic (1862), nearly 100 persons used to die every day in Oolah. Houses containing healthy individuals in the evening contained none but dead bodies the next morning. People who went to burn the dead came back ill of fever and died within a few hours. The number of the dead were so very great that there were none to remove them, and houses were found full of decomposing dead bodies of whole families.

I consider that the disease which, according to the *Indian Medical Gazette*, 1873 (Vol. VIII), converted some parts of the districts of Burdwan and Hooghly into a " valley of the shadow of death " into which if any human being, whether robust or weakly, well-nourished or the opposite, entered, he was almost certain to get an attack of fever, and get a very severe one, and, he might consider himself very fortunate if he escaped out of it with life, must have been, to some extent at least, due to malaria. It is questionable whether the fever that, according to Ward affected every

native in Burdwan, and afterwards, according to Metcalfe every human being resident in the town and which, according to Payne, affected the rich and poor alike, was all kala-azar. On the other hand, the disease that, according to Jackson, recurred in the same houses from year to year was more likely to be kala-azar.

The Epidemic Commission in 1864 described the deadly forms of the diseases as follows : "The disease is characterised in its deadly form by great general prostration, cerebral congestion and early collapse from which the patient, having no power to rally, is cut off in 36 hours to four or five days. During a first attack the head is the seat of congestion. The eyes are bloodshot and aching, the face is suffused, delirium early ensues and collapse terminating fatally in a few hours closed the scene. Next in urgency to the cerebral symptoms, we have to deal with a highly congested state of the thoracic viscera and with great difficulty of breathing; the air-tubes become loaded with mucus and death finally results from asphyxia." According to French, one common and dangerous form of Burdwan fever was that, after a few days of continued fever, violent vomiting and purging set in, followed by collapse, and in some cases death. In other cases that proved rapidly fatal, the symptoms varied very little from those of cholera. He describes three types of Burdwan fever—"the ordinary ague, which may be quotidian tertian and quartan, the mild remittent, and the malignant remittent." "The malignant remittent is the really fatal fever in Burdwan, although the mild remittent, by becoming congestive, may prove rapidly fatal." Greene points out : "There would appear to be two forms or aspects of the fever, viz., first, the virulent congestive form, remittent at first, with extreme prostration, with typhoid adynamic symptoms, contagious and very fatal; the second, the intense malarial intermittent with enlarged spleen and liver and protracted blood contamination, the one form
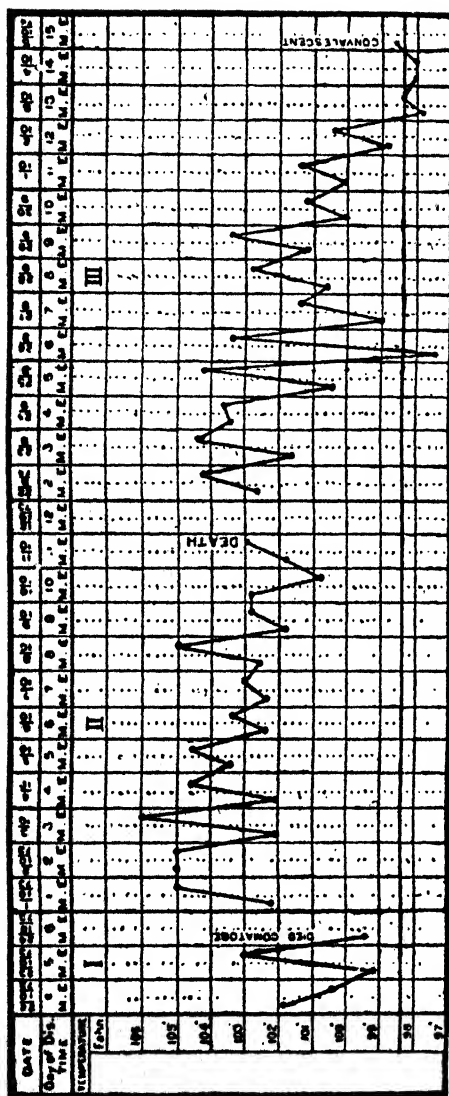
Three temperature charts of the old " Curdwan fever "

relapsing, contagious and very fatal and not amenable to quinine, the other, an intense intensified intermittent with extreme prostration of the vital powers during the cold stage of collapse, and contagious and curable by quinine." A similar account of acute cases of continued fever is given by Jackson. He states that "these acute cases are numerous enough; they constitute the fever."

I would now quote here a few charts from French's paper which, I consider, is one of the very few works that gives the temperature charts of the fever. As the book is extremely rare, I do not hesitate in reproducing some of his charts here.

It is evident that most of the acute types of the fever described above bear little or no resemblance whatever to kala-azar; either epidemic or sporadic. There can be no doubt that these were cases of intense malaria, which must have occurred as an epidemic in some of the affected places.

The pestilence that devastated Burdwan, Purbusthulee or Oolah must have been due to a great extent to an epidemic malarial fever. It would not, therefore, be correct to say that malaria did not form a part of the epidemic of Burdwan fever. It is impossible to say what proportion of the fever was due to malaria, but it certainly constituted a large proportion of the cases that died during an acute attack. It is possible that part at least, of the epidemic was due to an epidemic manifestation of endemic malaria that doubtless existed in Lower Bengal before the days of the epidemic. It is easy to understand in the present day, how endemic malaria became epidemic by the gradual silting up of the natural drainage outlets of a well-drained, healthy and prosperous tract of country. This must have occurred in Lower Bengal in the days of the epidemic, as Payne and Smith suggested in the seventies.

The endemic disease must, however, have been very mild, before the days of the epidemic, in many of the places

that were subsequently terribly affected, specially Burdwan. Before the days of the epidemic the district of Burdwan was noted for its healthiness, and the town of Burdwan was regarded as a sanitarium. It was even customary for persons suffering from chronic malaria to go to Burdwan where cures from the disease were common.

The census report for 1881 states that quarter of a century ago the district was considered one of the most salubrious in the province. If we turn to Hamilton's account of the district, we find that three-quarters of a century previous to the epidemic " there were few villages in Burdwan, in which there was not a school in which children are not taught to read and write ; there is no portion of territory in Hindoostan that can compare with it for productive agricultural value ;— in proportion to its size, it appears like a garden surrounded by wilderness." " Similarly the census report of the Nadia district for the year 1902 speaks of the district as "once famous as a health resort," though doubts have been thrown as to the correctness of this statement in the Bengal Gazetteers.

It would thus appear that cases of true malaria were few and far between in the Burdwan district before the days of the epidemic. The history of the malariousness of the district is to be traced to the epidemic of the Burdwan fever. That, not an inconsiderable number of cases in the Burdwan epidemic were purely cases of malarial origin is also proved by the fact that they got benefit, however temporary, from quinine. Dr. Roy thus writes : " The reputation which some of the quack medicines have attained in the cure of fever is owing to their containing quinine in fair proportion."

In Jamalpur, a pundit made his fortune by selling a mixture of his own composition, and as much as 100 bottles per day used to be sold in the fever season. Dr. Gupta's mixture received a very encouraging support. The atten-

dance at and reputation of a dispensary in the endemic district depended merely upon the quantity of quinine given than upon the skill and attention of the medical officers. Thus the daily attendance in one dispensary rose from 30 to 400, quinine was directly supplied for distribution.

Chevers writes that, all reliable facts that he was able to obtain combined to show that the Burdwan fever originated as a simple malarial fever quite amenable to quinine and ordinary treatment when taken in time.

There cannot thus be any doubt that a large number of cases of Burdwan fever were benefited by quinine. Another large number derived no benefit from the drug, which sometimes did harm. Thus Elliot pointed out that in acute cases the drug was useless and even harmful and tended to increase internal congestion. He further points out that intercurrent with the epidemic disease there were many cases of the ordinary endemic fever in an intensified form more or less amenable to quinine, but certain to recur at some periods within a month and as certainly followed by enlargement of the spleen.

It will thus be seen that the Burdwan fever was partly an epidemic malarial fever, i.e., malarial fever causing a great rise in the death curve. The relapsing cases were also partly cases of chronic malaria. The chronic cases that, according to Elliot and others, got marked benefit from quinine or were cured by change of place must have been cases of true malaria. On the other hand, those that suffered from recurring attacks of fever and in the long run had enormously enlarged spleen and liver and were not benefited by quinine, and finally suffered from œdema of the extremities and cancrum oris were very likely cases of kala-azar.

If we study the sequelæ of the Burdwan fever, this fact becomes evident. Roy describes them as follows: " The first and most frequent in order is enlargement of the spleen. It varies considerably in size, from being just perceptible

under the costal cartilages to filling up the whole abdomen. From a distance the pot-bellied feature with distended veins on the surface of the abdomen gives an appearance of dropsy. There may be enlargement of the liver. There may be obstruction to the portal circulation ending in ascites. There may be large prominent veins on the surface of the abdomen. Dropsy is the sequel with which these cases take a fatal turn.

The lean emaciated limbs and haggard countenance seen on a bloated trunk gives a most unsightly appearance. Unless timely treated with tonics and nourishment, cancrum oris makes its appearance and puts an end to the patient's miserable existence. It is frequently accompanied with dysentery. There is a watery state of the blood and there may be hæmorrhagic diathesis. Bleeding from the nose and gums is a very common complication. There may be bleeding from the mouth or rectum."

Having attempted to prove from clinical evidence that the Burdwan epidemic was to a great extent one of malarial origin, I pass on to show the same by comparing the mortality curve from *fever* in the district of Burdwan during the epidemic with that of Goalpara during the kala-azar epidemic in the latter district. If we compare the two curves, we find the following differences : —

(1) In the Burdwan epidemic, the mortality was lowest about June. In Goalpara the highest rise in the mortality curve was generally in June and sometimes in December. In some years there was a double rise, one about June and another about December. In the Burdwan epidemic the largest mortality was about December.

(2) There was a very high rise in the mortality curve every year in the Burdwan epidemic about December, while in Goalpara it was very high every month. The proportion of the highest to the lowest mortality was 12 to 1 in the Burdwan epidemic, while it was less than 2 to 1 in the Goalpara epidemic.
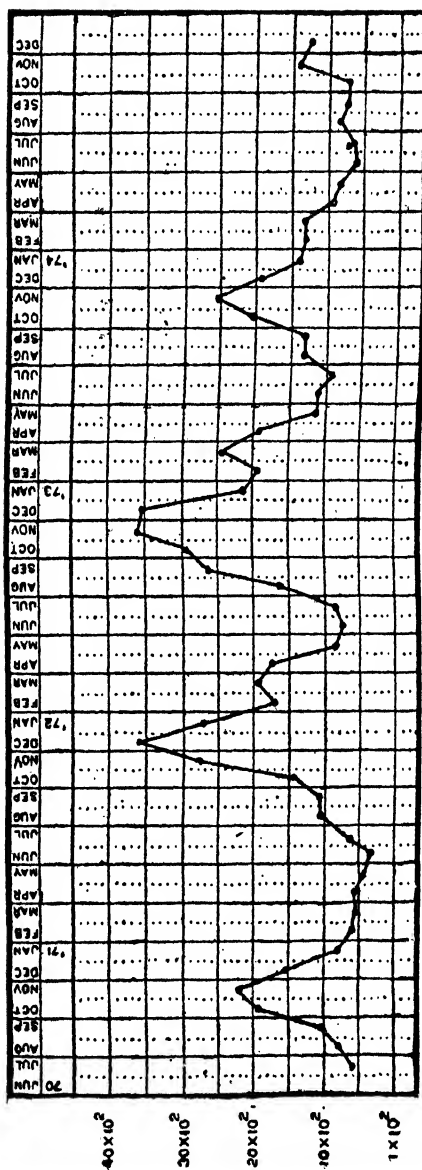
Mortality curve in the " Burdwan Epidemic "
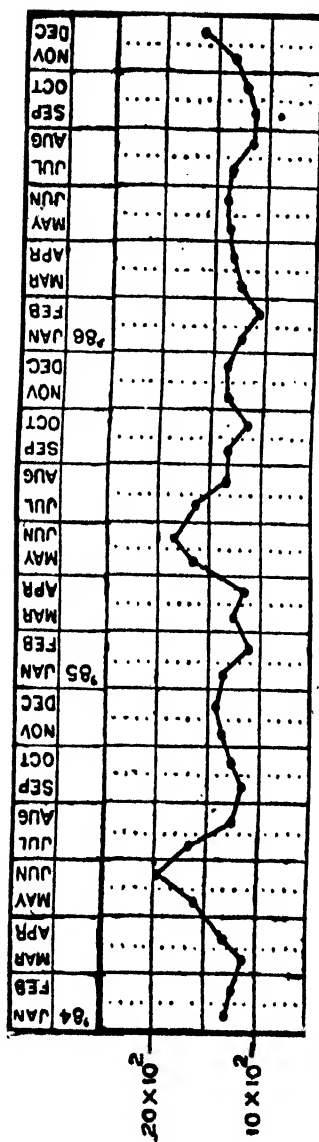
Mortality curve in the " Goalpara Epidemic "
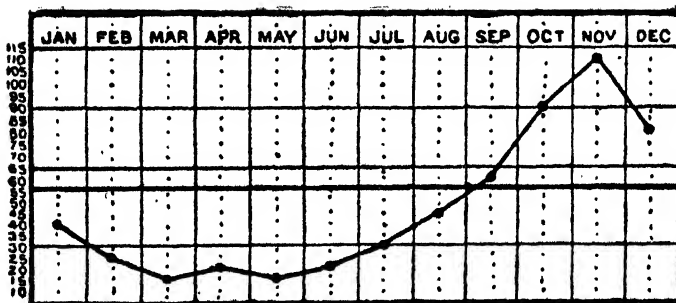
Malaria curve in Bengal (Rogers and Megaw)

(3) The period of the highest mortality in the Burdwan epidemic was exactly coincident with that of the highest rise in the malaria curve in Bengal, *i.e.*, about December.

It is thus evident that there was an epidemic of two diseases during the outbreak of Burdwan fever. The severe cases described by French were mostly cases of malaria (probably malignant tertian fever), while those that constituted the large majority of cases observed by Jackson were cases of kala-azar. In most cases the two diseases travelled together, but not always so. Jackson thought that the disease travelled along roads and lines of traffic and intercommunication. On the other hand, French thought that the disease extended chiefly and was worst along rivers, khals, and streams. To-day we can reconcile both these views; French was concerned mostly with cases of epidemic malarial fever, while Jackson's cases were mostly cases of kala-azar.

The effect of the epidemic of the Burdwan fever on the health of the district is an interesting study. At the present day the district of Burdwan is notorious for malaria. There are also cases of kala-azar that come to us for treatment.

There is thus much truth when French wrote, that the Burdwan fever " differed only in degree or virulence from the ordinary autumnal malarious fevers of Bengal." Whether kala-azar appeared as a new disease in Burdwan during the epidemic, or whether it appeared as an epidemic manifestation of a sporadic disease, must always remain a mystery.

The epidemiology of malaria seems, therefore, to have been closely connected with that of kala-azar in the Burdwan epidemic. This fact is not purely of academic interest but will be of greatest value to those who are concerned with the study of epidemiology of malaria in Bengal. Whether

there was a causal relationship between the two diseases is a subject of the greatest interest to the scientific enquirer.

In conclusion, I would express my deep indebtedness to Mr. P. Dias of the Imperial Record office for furnishing me with many publications in connection with the Burdwan Epidemic.

BURDWAN FEVER

References :—

(1) Elliott.— Report on Epidemic Remittent and Intermittent fevers occurring in parts of Burdwan and Nudia Districts, 1863.

(2) Report of the Epidemic Commission, 1864.

(3) Green.—Report on the nature and history of fever in the Districts of Lower Bengal, 1868.

(4) No. 50 of 25th May, 1871 from Ward, Magistrate of ˙Burdwan (referred to in French's paper).

(5) No. 394 of the 30th December, 1871, from Payne, Offg. Sanitary Commissioner, Bengal (Supplement to the *Calcutta Gazette*, January 10th, 1872).

(6) No. 960 of 27th December, 1871 from Browne, Inspector-General of Civil Hospitals (Supplement to the *Calcutta Gazette*, January 10th, 1872).

(7) Gupta.—Report on the Burdwan Fever, 1872.

(8) No. 267 of 16th May, 1872, from Buckland, Commissioner of Burdwan (Supplement to the *Calcutta Gazette*, June 5th, 1872). No. 367 of 6th July, 1872 from Buckland (Suplement to *Calcutta Gazette*, July 24, 1872).

(9) Report on Sanitary Measures in India, 1872.

(10) Jackson.—Report on Burdwan Fever, 1873.

(11) Lyon.—Burdwan Fever.

(12) Verchere.—Extracts from a diary kept in Burdwan, 1873-74, *Indian Medical Gazette*, 1873-74.

(13) French.—Endemic Fever in Lower Bengal, 1874.

(14) French.—Endemic Fever in Lower Bengal, 1875.

(15) Ray.—Burdwan Fever, 1876.

(16) Chevers--Diseases of India, 1886.

(17) Hunter.—Imperial Gazetteer of India.

(18) Buckland.—Bengal under the Lieutenant-Governors.

(19) Bengal District Gazetteers (Burdwan and Nadia).

(20) Rogers.—The Lower Bengal (Burdwan) epidemic
        reviewed, 1897.

(21) Rogers.—Fevers in the Tropics, 1908.

(22) Transactions of Bombay Medical Congress, 1909.

(23) Report of the Sanitary Commissioner of Bengal,
        1874.

(24) Mullick.—*Nuddia Kahini*, 1910.

(25) *Indian Medical Gazette*, 1871-1875.

Chart I.—Three cases—Temperature Charts. •

Chart II.—Mortality Curve from " Fever " during the
        Burdwan Epidemic (Burdwan District).

Chart III.—Mortality Curve from " Fever " in Goalpara
        district during the Kala-azar Epidemic.

Chart IV.—Seasonal prevalence of Malaria in Bengal
        (Rogers' and Megaw's Statistics).

CERTAIN OBSERVATIONS ON THE MECHANISM OF QUININE HÆMO-GLOBINURIA IN MAN

Part I.

In this paper, no attempt is made to investigate the ætiology or mechanism of hæmolysis in black-water fever, but only to discuss, so far as the present research elucidates it, the mechanism of hæmoglobinuria observed in certain individuals whenever quinine is administered to them.

The observations recorded below were made in a patient who developed hæmoglobinuria each time quinine was administered to him. As the case is of unusual interest, we give a brief history of the case.

Patient, named Maitra, was admitted, on 4th September, 1924, into the medical ward of one of us, with history of fever and a recent attack of hæmoglobinuria following administration of quinine. On admission, his spleen was found extending five inches below the costal arch. Blood examination : R. B. C.—1,880,000, W. B. C.— 2,500, Hb.—35 per cent. Polymorphonuclears—28 per cent. Lymphocytes—58 per cent. Large Mononuclears—13 per cent. Eosinophiles—1 per cent. No malarial parasites. Blood culture on NNN medium showed the presence of leishmania flagellates. Patient was treated with urea stibamine. After 10 injections, the spleen could not be felt below the costal margin, and the blood culture made on NNN medium was found negative ; the fever disappeared and there was marked improvement in his

general condition.   Three months after treatment with urea stibamine, W. B. C. count was 7,100 and peripheral blood culture on NNN medium proved negative.

I.   Attacks of hæmoglobinuria after administration of quinine in the hospital

(i)   On 12th January, 1925, patient was given 5 grains of quinine bihydrochloride at 10 a.m.   Hæmoglobinuria developed at 11 a.m. which lasted up to 1 p.m. next day.

(ii)   On 19th March, 1925, patient was given 5 grains of quinine bihydrochloride at 11-30 a.m.   Hæmoglobinuria developed after 45 minutes and lasted up to 9 a.m. next day.

(iii)   On 14th May, 1925, patient was given 5 grains of quinine bihydrochloride at 9-50 a.m.   Hæmoglobinuria developed at 1 p.m. and lasted up to 12 noon next day.

(iv)   On 7th June, 1925, patient was given 5 grains of quinine bihydrochloride at 8-30 a.m.   Hæmoglobinuria developed at 11 a.m. and lasted up to 12 noon next day.

II.   Relation of hæmoglobinuria to malaria in the present case

The patient's blood was examined for ten successive days for the presence of malarial parasites both by the thin film as well as by the thick film method.   No malarial parasites could be detected.   No quinine was given before and during the periods of observation.

III.   The action of quinine on patient's red corpuscles

We made investigations to determine whether the red corpuscles of the patient were more easily vulnerable to quinine than those of individuals not susceptible to quinine hæmoglobinuria.   We quote here the effect of quinine bihydrochloride on red corpuscles of a series of individuals not susceptible to quinine hæmoglobinuria from a paper

of ours published in the *Biochemical Journal* (1921,
Vol. XV, No. 4, *corrected slip*) :—

Strength of quinine bihydrochloride giving rise to commencing hæmolysis when a suspension ' of red corpuscles is added to quinine bihydrochloride solution

Case No.	1	...	...	...	·04	per cent.
,,	2	...	...	...	·05	,,
,,	3	...	...	...	·055	,,
,,	4	...	...	...	·06	,,
,,	5	...	...	...	·05	,,
,,	6	...	...	...	·085	,,
,,	7	...	...	...	·080	,,
,,	8	...	...	...	·055	,,
,,	9	..	...	...	·085	,,
.,	10	...	...	...	·06	,,
,,	11	..	...	...	·045	,,
,,	12	...	...	...	·035	,,
,,	13	...	...	...	·006	,,
,,	14	...	...	...	·055	,,
,,	15	...	...	...	·070	,.
,,	16	...	...	...	·08	,,
,,	17	...	...	...	·055	,,
,,	18	...	...	...	·065	,,

In the present case, commencing hæmolysis took place
with ·065 per cent of quinine bihydrochloride. It will thus
be seen that the red corpuscles of this patient were not more
vulnerable to quinine bihydrochloride than those of a series
of individuals none of whom had any tendency to quinine
hæmoglobinuria.

IV.   The action of the serum of the patient on the red corpuscles of other individuals not susceptible to quinine hæmoglobinuria and vice versa

No difference was observed in this respect. The red
corpuscles of the patient behaved with the serum of other
individuals in the same way as those of the latter did towards
the serum of the patient.

V. Experiments to determine whether any hœmolysin was developed in the patient's serum after administration of quinine

The following series of experiments were made to determine the point :

Time	Experiments	Observation	Experiments	Observation
10-40 a.m.	Blood taken five minutes before administration of quinine.	Serum not tinged with blood.	...	...
10-45 ,,	Five grains of quinine bihydrochloride dissolved in one ounce of water administered by the mouth.	...	...	...
11-15 ,.	Blood taken	Serum not tinged with hæmoglobin.	(1) Serum (2 c.c.) +corpuscles from blood taken at 10-40 a.m. (incubated at 37° C. for one hour).	No hæmolysis
			(2) Serum (2 c.c.) +same corpuscles as above+1 c.c. complement (incubated for one hour).	Do.
12 noon	Blood taken	Serum very slightly tinged with hæmoglobin.	(1) Same as above, No. 1.	Do.
			(2) Same as above, No. 2.	Do.
12 45 p m.	Blood taken	Serum not much tinged with hæmoglobin.	(1) Same as above, No. 1.	Do.
			(2) Same as above, No. 2.	Do.
12-45 ,	Urine examined	Hæmoglobinuria set in for the first time.	(1) Experiments same as before.	Do.
			(2) Same as before.	Do.
1-15 ,,	Do.	Hæmoglobinuria increasing.	Do.	Do.
6 ,,	Do.	Hæmoglobinuria persisting.	...	...
12 ,,	Do.	Do.	...	...
7 a.m	Do.	Very little hæmoglobinuria.	...	...
11 ,,	Do.	Do.	...	...
1 p.m.	Do.	No hæmoglobinuria.	...	...

It will thus be seen that no hæmolysin was developed in the peripheral blood of the patient during the stages when active hæmolysis took place after administration of quinine.

VI.   Observations to determine the seat of active hæmolysis after administration of quinine

The following experiment was performed to determine this point :

Patient was given 5 grains of quinine bihydrochloride by the mouth at 8-50 a.m. on 7th June, 1925. Hæmoglobinuria was noticed at 11 a.m.

The peripheral blood of the patient was examined immediately after hæmoglobinuria appeared. Blood was also taken from the liver at the same time. The following remarkable observations were made :

By comparing the colour of the dissolved hæmoglobin in the serum with that in the urine, by putting them into two Haldane hæmoglobinometer tubes, it was found that the amount of dissolved hæmoglobin in the peripheral blood was the same as in the urine, but no quantitative estimation could be made on account of the very small amount of dissolved hæmoglobin present. In the liver, on the other hand, the amount of dissolved hæmoglobin in 20 c.mm. of the serum obtained from the blood from liver puncture showed an amount of hæmoglobin which amounted to 10 per cent of hæmoglobin in the hæmoglobinometer scale.

It will thus be seen that while the amount of dissolved hæmoglobin in the peripheral blood at the commencement of active hæmolysis was so small as could not be determined by the hæmoglobinometer, the amount of dissolved hæmoglobin in 20 c. mm. of the serum obtained from the blood from the liver at this stage was 10 per cent. It is thus evident that the liver was *at least* one of the internal organs in which active hæmolysis was taking place when the person was suffering from quinine hæmoglobinuria.

An explanation may be offered of this remarkable phenomenon.

When quinine is administered by the mouth, it evidently passes through the liver after absorption. If for some reason the whole quinine remains in the liver for a longer time than it does in a normal individual, it is likely that a concentration of quinine may take place in the liver, even with only 5 grains of quinine, at which concentration the red corpuscles present in the blood inside the liver may be hæmolysed.

The mechanism of quinine hæmoglobinuria therefore appears to be due to a concentration of the drug in the liver of the susceptible individuals greater than what occurs normally, with the result that the concentrated quinine dissolves the red corpuscles present in the blood of the liver resulting at first in hæmoglobinæmia and subsequently hæmoglobin·uria. Why quinine remains inside the liver of certain individuals for a longer time than in others is a subject for research. Perhaps it remains adsorbed with an undiscovered substance in the liver which is now the subject for our further research.

Remarks

1. In individuals who have a tendency to quinine hæmoglobinuria, the red corpuscles are not more vulnerable to quinine than those of other individuals who have no such tendency.

2. No hæmolysin was discovered in the peripheral blood during the active stages of quinine hæmoglobinuria.

3. We have demonstrated that during the active stages of quinine hæmoglobinuria, the process of hæmolysis takes place at least in one of the internal organs, namely, the liver.

4. There is nothing in the patient under our observation to indicate that this tendency to hæmoglobinuria was due to attacks of malaria.

5. The patient was suffering from kala-azar and although he was cured of his disease by urea stibamine,

tendency to quinine hæmoglobinuria was not diminished after the cure was effected.

6.   We suggest that in individuals susceptible to quinine hæmoglobinuria, quinine probably remains adsorbed with an *unknown* substance in the liver for prolonged periods giving rise to a concentration of the drug, at which quinine hæmolyses red corpuscles in the test tube. It is a known fact that when quinine is administered to any individual, it does tend to concentrate in the liver; but whether hæmolysis will take place in the liver will depend upon (1) the concentration of quinine in the liver and (2) the period during which such a concentration is maintained in the organ.

CERTAIN OBSERVATIONS ON THE MECHANISM OF QUININE HÆMOGLOBINURIA IN MAN

Part II

In the *Indian Journal of Medical Research*, October 1925, Brahmachari and Sen gave records of certain observations on the mechanism of quinine hæmoglobinuria in man. Since then, I have made observations on a case of black-water fever, in which the mechanism of hæmoglobinuria was found to be similar to that of quinine hæmoglobinuria.

Patient, æt. 14, came under my observation with history of hæmoglobinuria for about four days which appeared 6 hours after oral administration of 8 grains of quinine, increasing during paroxysms of fever and diminishing during periods of apyrexia. The spleen was hard—extending 2 inches below the costal arch. The fever was of an intermittent type with double rise during 24 hours. When I saw him for the first time he had marked hæmoglobinuria, but no examination of the hepatic and peripheral blood could be madeon this occasion. I saw him again 24 hours later when the hæmoglobinuria was passing off. All the observations noted below were made on this occasion :—

R. B. C.—2,000,000, W. B. C.—2,812, Hb.—40 per cent, Polynuclears—56 per cent, Eosinophiles—2 per cent, Small Mononuclears—36 per cent, Large Mononuclears—

6 per cent. No malarial parasites. L. D. bodies found in smears from the liver and on culture of the peripheral blood.

The patient was asked to pass urine and at the same time blood from the liver and a vein at the bend of the elbow was taken. The following observations were made :—

(1) Blood from liver—showed distinct signs of hæmolysis.

(2) Peripheral blood—very faint hæmolysis.

(3) Urine—very faint hæmoglobinuria.

In other words, even when the hæmoglobinuria was passing off, the presence of hæmolysed blood in the liver was greater than in the peripheral blood and the urine. Thus the liver was at least one of the internal organs in which active hæmolysis was taking place as in the case of quinine hæmoglobinuria recorded in my first paper.

OBSERVATIONS

The following are the points of interest in the case :—

(1) Patient was accustomed to take quinine for attacks of malarial fever from which he was suffering from time to time, but this was the first occasion he had hæmoglobinuria.

(2) The present attack of hæmoglobinuria was of an intermittent nature, increasing during heights of fever and diminishing during apyrexia.

(3) During the stage of active hæmolysis, hæmolysis was going on in the liver just as in the case of quinine hæmoglobinuria.

(4) Though the patient gave a history of malarial attacks, he was now suffering from kala-azar.

This is the second case in which hæmoglobinuria was manifested in a case of kala-azar.

STUDIES IN BLACK-WATER FEVER

Part I. Variation in the intensity of hæmoglobinuria
following administration of quinine by regulation
of the dosage of quinine in a susceptible
individual

Whatever may be the mechanism of black-water fever, there is no doubt that there are many cases in which quinine is a determining factor and its administration • may be followed by severe hæmoglobinuria and sometimes with disastrous results, while in other cases, quinine has little or no influence upon the disease.   The disease may there-fore be classified under two heads :

1. Quinine-intolerant type. This includes cases in which hæmoglobinuria is precipitated by the administration of quinine.   It can be subdivided into two groups :

(*a*) *Mild*—in which the hæmoglobinuria stops as soon as
quinine is discontinued.

(*b*) *Severe*—in which hæmoglobinuria persists after the
discontinuance of quinine and the condition may
end fatally.

2. Quinine-tolerant type. This includes cases in which hæmoglobinuria has little or no relationship with administra-tion of quinine.

We describe here a case of malarial fever in which it was possible experimentally to bring about hæmoglobinuria, the degree of which could be varied by increasing or diminishing the dosage of quinine. The case was intolerant to almost any dose of quinine. The patient was a boy of six years añd was the son of a medical man who stated that the boy used to get attacks of hæmoglobinuria, jaundice and high rise of temperature each time quinine was administered to him. When he came under observation of one of us (U. N. B.) he was suffering from malignant tertian infection. The spleen was enlarged and there was presence of malignant tertian parasites in the blood.

1. One-twelfth grain of quinine bihydrochlor or quinine base or euquinine twice a day was first tried, but this gave rise to frequent yawning, weakness and faint hæmoglobinuria which could be demonstrated in the urine by means of the spectroscope.

2. One grain of quinine bihydrochlor or quinine base or euquinine gave rise to pain in the abdomen and limbs, jaundice, well-marked hæmoglobinuria and distinct rise of temperature.

3. Two and a half grains of quinine bihydrochlor was followed by quick and feeble pulse, intense pain in the limbs and abdomen, great prostration, marked jaundice, marked hæmoglobinuria and high fever (temperature-105°F). The symptoms were somewhat alarming, but fortunately subsided in forty-eight hours.

4. Five grains of quinine bihydrochlor was followed, as stated by the father of the patient, by intensive hæmoglobinuria, very severe prostration, drowsiness and the condition was almost fatal.

5. Administration of large doses of sodii citras, and intravenous injection of 1˙5 to 2˙5 c.c. of a 10 per cent solution of calcium chloride brought about a slight increase of tolerance to quinine. Still the patient could not bear

2·5 grains of quinine bihydrochlor given in divided dose
of 0·5 grain each in spite of his having 10 grains of sodii
citras four times a day, and daily intravenous injection of
2·5 c.c. of a 10 per cent solution of calcium chloride and
oral administration of 5 m. of adrenaline solution thrice a
day.

The treatment of an attack of malarial fever in cases
of this type is evidently one of the most difficult problems
in tropical medicine. On the one hand, withdrawal of
quinine may give rise to the persistence of the
parasites in the blood which may lead to symptoms of
pernicious malaria ending in death ; on the other hand,
administration of quinine may bring about untoward
or even fatal results. We are unable to agree with those
observers who consider that cases of hæmoglobinuria following
administration of quinine are not severe and that their
prognosis is usually good.

*Part II.   Observations on the action of quinine on
erythrocytes in cases of black-water fever*

In order to observe the action of quinine on erythrocytes,
about 3 c.c. of blood were taken from the vein in 5 c.c. of
a 2 per cent sodium citrate solution in normal saline. The
blood was then washed three times in normal saline and
a 5 per cent suspension of the erythrocytes was made.
After mixing the blood with the quinine solution, the test
tubes containing the mixture were kept in an incubator
at 40°C. for one and one-half hours.

In testing the action of a quinine solution of a particular
strength, a solution of the double strength was prepared
and mixed with an equal part of 5 per cent suspension of
erythrocytes. In all the tables, only the resulting strength
of quinine solution is given.

5—767B(II)

Case 1.   Patient was admitted into hospital with history of remittent type of fever for about eleven days.   He was given intramuscular injections of 5 grains of quinine bihydrochlor on May 23, May 25, and May 26, 1931. Patient had hæmoglobinuria before he was admitted into hospital, 'and it continued up to May 30, 1931.   At the time of admission he was somewhat drowsy, very much prostrated, extremely anæmic and jaundiced.   Examination of blood on May 30, showed : red blood cells, 840,000; white blood cells, 5000; polymorphonuclears, 66 per cent; small mononuclears, 24 per cent; large mono- nuclears, 10 per cent; eosinophiles, nil.   No malarial parasites were present in the peripheral blood.   Urine showed the presence of dissolved hæmoglobin, bile pigments and casts.   There were no red blood cells in the urine. Patient died on June 5, 1931.   No *post-mortem* was allowed by the relatives of the patient.

Blood was tested with quinine hydrochloride solution on the fourth day after stoppage of hæmoglobinuria (table 1).

TABLE 1

Action of quinine hydrochloride on the erythrocytes

Strength of qui- nine hydrochlor	Blood of black-water fever case	Normal blood
per cent		
0·05	No hæmolysis	No hæmolysis
0·1	Commencing hæmolysis	No hæmolysis
0·2	Slight hæmolysis	No hæmolysis
0·25	Slight hæmolysis	Commencing hæmolysis
0·3	Moderate hæmolysis	Slight hæmolysis
0·35	Marked hæmolysis	Moderate hæmolysis
0·4	Almost complete hæmolysis	Marked hæmolysis

TABLE 2

Action of quinine on the erythrocytes

Strength of quinine hydrochlor	Blood of black-water fever case		Normal blood
	Before injection of quinine bihydrochlor	After injection of quinine bihydrochlor and during the presence of active hæmolysis	
per cent			
0·05	No hæmolysis	No hæmolysis	No hæmolysis
0·1	No hæmolysis	No hæmolysis	No hæmolysis
0·15	No hæmolysis	No hæmolysis	No hæmolysis
0·2	No hæmolysis	No hæmolysis	No hæmolysis
0·25	No hæmolysis	*Commencing hæmolysis*	No hæmolysis
0·3	*Commencing hæmolysis*	Slight hæmolysis	No hæmolysis
0·35	Slight hæmolysis	Moderate hæmolysis	*Commencing hæmolysis*
0·4	Moderate hæmolysis	Marked hæmolysis	Slight hæmolysis
0·45	Marked hæmolysis	Almost complete hæmolysis	Moderate hæmolysis

Case 2. Patient was admitted into hospital on July 23, 1931, with history of hæmoglobinuria on the previous day. At the time of his admission there was no hæmoglobinuria. Examination of peripheral blood on July 24, showed presence of benign tertian parasites.

On July 24, at 11-00 a.m. the patient was given an intramuscular injection of 8 grains of quinine bihydrochloride. Hæmoglobinuria started two hours after its administration and disappeared after forty-eight hours. A sample of his blood was taken one hour before the administration of quinine, and another during active hæmolysis, *i.e.*, two and a half hours after hæmoglobinuria had started or five hours and a half after administration of quinine (table 2).

TABLE 3

Strength of quinine solution	Action on blood from black-water fever case
per cent	
0·05	No hæmolysis
0·1	No hæmolysis
0 2	No hæmolysis
0 25	No hæmolysis
0·3	*Commencing hæmolysis*
0·35	Slight hæmolysis
0 4	Moderate hæmolysis
0·45	Marked hæmolysis

TABLE 4

Strength of quinine bihydrochloride	Blood of black-water fever case	Blood of non-black-water fever case
per cent		
0·05	No hæmolysis	No hæmolysis
0·1	*Commencing hæmolysis*	No hæmolysis
0·15	Slight hæmolysis	*Commencing hæmolysis*
0·2	Marked hæmolysis	Slight hæmolysis
0·25	Marked hæmolysis	Marked hæmolysis
0·3	Marked hæmolysis	Marked hæmolysis
0·35	Marked hæmolysis	Marked hæmolysis
0·4	Marked hæmolysis	Marked hæmolysis
0·45	Marked hæmolysis	Marked hæmolysis

On August 6, the patient's erythrocytes were tested again in the same way as before. There was no active hæmolysis at this time. The results obtained were identical with those above as shown in table 3.

No quinine was given to the patient, after the first appearance of hæmoglobinuria in the wards, till the re-appearance of the parasites on August 23, 1931. Spleen was now found enlarged to about 3 fingers' breadth below the costal margin.

On August 24, 2 grains of quinine bihydrochlor were given orally thrice a day, the last dose being taken at 7-00 p.m. Hæmoglobinuria appeared at 9-30 a.m. on

August, 25. Blood was taken on the same day after appearance of hæmoglobinuria for the second time and this time the erythrocytes were tested with solution of quinine bihydrochloride instead of hydrochloride as in the previous experiments. At the same time erythrocytes of a non-black-water fever case were tested in the same way with the results shown in table 4.

OBSERVATIONS

The following conclusions may be made from the above experiments :

1. The erythrocytes of cases of black-water fever following administration of quinine are slightly more vulnerable to quinine than those of normal individuals.

2. There is no difference in the vulnerability of erythrocytes of cases of black-water fever to quinine : (a) before hæmoglobinuria, (b) during active hæmolysis after administration of quinine, and (c) after the disappearance of the hæmoglobinuria.

3. The increased vulnerability of erythrocytes of cases of black-water fever to the action of quinine is so very slight that it can not account for the black-water fever that may follow quinine administration, and, therefore, whatever may be the mechanism of hæmoglobinuria following administration of quinine, there is no evidence to show, so far as experiments in vitro are concerned, that it is due to any direct action of quinine upon the erythrocytes of susceptible individuals.

STUDIES IN BLACK-WATER FEVER

Parts I and II of our papers on "Studies in Black-water Fever" have been published in the *American Journal of Tropical Medicine*, Vol. XII, No. 2, March, 1932. In these papers we observed that whatever may be the mechanism of hæmoglobinuria, following administration of quinine, there is no evidence to show, so far as experiments *in vitro* are concerned, that it is due to any *direct* action of quinine upon the erythrocytes of susceptible individuals.

The following experiments were made by us to determine the action of quinine bihydrochlor and quinine hydrochlor in various strengths *in vitro* upon a hæmolytic system.

Observation

In our previous papers we noted the hæmolytic action of quinine *in vitro*. The present paper shows that solutions of quinine bihydrochlor, or hydrochlor, in strengths not having any direct hæmolytic action on red corpuscles, inhibit hæmolysis in a hæmolytic system. If, therefore, in the hæmolysis of black-water fever following administration of quinine, a hæmolytic system plays a part, then quinine hydrochlor or bihydrochlor would inhibit hæmolysis in that system, so far as experiments *in vitro* prove. Whether, at the same time, quinine helps in the development of a hæmolytic system in a susceptible individual, we are at present unable to say.

The management of the hæmoglobinuria following administration of quinine in susceptible individuals suffering from malarial fever is a very difficult problem. Such cases are much more common than cases of anaphylaxis following anti-diphtheritic or anti-tetanic injection, and cannot be lightly viewed in consideration of their commonness and dangerous nature. It is not too much to state that such cases do occur in the practice of most physicians in the tropics who have to treat cases of malarial fever.

REFERENCE

The American Journal of Tropical Medicine, Vol. XII, No. 2, March, 1932.

Experiments with Quinine Bihydrochloride

TABLE 1 (a)

N.B.—In these experiments, quinine bihydrochlor solution of varying strength was first mixed with complement and then put in the incubator for half an hour and then treated with haemolysin and red corpuscles

Amount and strength of Q. bihydrochlor solution	Amount of complement	Amount of normal saline	Incubation for ½ hour at 37° C.	Amount of haemolysin	Amount of 5% R.B.C. suspension	Incubation for ¼ hour at 37° C.	Percentage of inhibition of haemolysis		
							Expt. No. 1.	Expt. No. 2.	Expt. No. 3
0·2 c.c. of 0·4%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c.c.		30%	50%	30%
0·2 c.c. of 0·3%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c.c.		—	50%	30%
0·2 c.c. of 0·2%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c.c.		30%	50%	30%
0·2 c.c. of 0·15%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c.c.		—	50%	30%
0·2 c.c. of 0·1%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c.c.		30%	50%	30%
0·2 c.c. of 0·075%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c.c.		30%	30%	30%
0·2 c.c. of 0·05%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c.c.		30%	30%	30%
0·2 c.c. of 0·0375%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c.c.		—	30%	30%
0·2 c.c. of 0·025%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c.c		—	30%	30%

TABLE 1 (b)

N.B.—In these experiments, quinine bihydrochlor solution of varying strength was first mixed with haemolysin and then put in the incubator for half an hour and then treated with complement and red corpuscles

Amount and strength of Q. bihydrochlor solution	Amount of haemolysin	Amount of normal saline	Amount of complement	Amount of 5% R.B.C. suspension	Percentage of inhibition of haemolysis	
					Expt. No. 1	Expt. No. 2
0.2 c.c of 0.075%	+ 0.2 c.c.	+ 0.2 c.c.	+ 0.2 c.c.	+ 0.2 c.c.	30%	30%
0.2 c.c. of 0.1%	+ 0.2 c.c.	+ 0.2 c.c	Incubation for ½ hour at 37° C	+ 0.2 c.c.	Incubation for ½ hour at 37° C.	30%	30%
0.2 c.c. of 0.2%	+ 0.2 c.c.	+ 0.2 c.c.	+ 0.2 c.c.	+ 0.2 c.c.	30%	30%
0.2 c.c. of 0.3%	+ 0.2 c.c.	+ 0.2 c.c.	+ 0.2 c.c.	+ 0.2 c.c.	30%	30%

TABLE 1 (c)

N.B.—In these experiments, quinine bihydrochlor solution of varying strength was mixed with complement, normal saline, haemolysin, and red corpuscles at the same time and then put in the incubator for half an hour

Amount and st.ength of Q. bihydrochlor. solution	Amount of complement	Amount of normal saline	Amount of haemolysin	Amount of 5% R.B.C. suspension	Percentage of inhibition of haemolysis
0.2 c.c. of 0.075%	+ 0.2 c.c.	+ 0.2 c.c.	+ 0.2 c.c.	+ 0.2 c.c.	30%
0.2 c.c. of 0.1%	+ 0.2 c.c.	+ 0.2 c.c.	+ 0.2 c.c.	+ 0.2 c.c	Incubation for ½ hour at 37° C.	30%
0.2 c.c. of 0.2%	+ 0.2 c.c.	+ 0.2 c.c.	+ 0.2 c.c.	+ 0.2 c.c.	30%
0.2 c.c. of 0.3%	+ 0.2 c.c.	+ 0.2 c.c.	+ 0.2 c.c.	+ 0.2 c.c.	30%

TABLE 2 (a)

N.B.—In these experiments, quinine hydrochlor solution of varying strength was first mixed with complement and then put in the incubator for half an hour and then treated with hæmolysin and red corpuscles

Amount and strength of Q. hydrochlor solution	Amount of complement	Amount of normal saline	Incubation for ½ hour at 37° C.	Amount of hæmolysin	Amount of 5% R.B.C. suspension	Incubation for ½ hour at 37° C.	Percentage of inhibition of hæmolysis		
							Exp No. 1	Exp. No. 2	Exp. No. 3
0·2 c c of 0·4%	+0·2 c c	+0·2 c.c.		+0·2 c c	+0·2 c.c.		—	50%	30%
0·2 c c of 0·3%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c.c.		—	5 %	30%
0·2 c c. of 0·2%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c.c.		—	30%	30%
0·2 c.c. of 0·15%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c c		50%	30%	30%
0·2 c.c. of 0·1%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c c		—	30%	30%
0·2 c.c. of 0·075%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c.c.		30%	30%	30%
0·2 c.c. of 0·05%	+0·2 c.c.	+0·2 c.c.-		+0·2 c.c.	+0·2 c c.		—	30%	30%
0·2 c c. of 0·0375%	+0·2 c.c.	+0 2 c.c.		+0·2 c.c.	+0·2 c.c.		—	30%	30%
0·2 c.c. of 0·025%	+0·2 c.c.	+0·2 c.c.		+0 2 c.c.	+0·2 c.c.		—	30%	30%

TABLE 2 (b)

N.B.—In these experiments, quinine hydrochlor solution of varying strength was first mixed with haemolysin and then put in the incubator for half an hour and then treated with complement and red corpuscles

Amount and strength of Q. hydrochlor solution	Amount of haemolysin	Amount of normal saline		Amount of complement	Amount of 5% R.B.C. suspensic.n		Percentage of inhibition of haemolysis	
							Expt. No. 1	Expt. No. 2
0·2 c.c. of 0·075%	+0·2 c.c.	+0·2 c.c.	Incubation for ½ hour at 37° C.	+0·2 c.c.	+0·2 c.c.	Incubation for ½ hour at 37° C.	30%	30%
0·2 c.c. of 0·1%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c.c.		30%	30%
0·2 c.c. of 0·2%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c.c.		30%	30%
0·2 c.c. of 0·3%	+0·2 c.c.	+0·2 c.c.		+0·2 c.c.	+0·2 c.c.		30%	30%

TABLE 2(c)

N.B.—In these experiments, quinine hydrcchlor solution of varying strength was mixed with complement, normal saline, haemolysin, and red corpuscles at the same time and then put in the incubator for half an hour

Amount and strength of Q. hydrochlor solution	Amount of complement	Amount of normal saline	Amount of haemolysin	Amount of 5% R.B.C. suspension		Percentage of inhibition of haemolysis
0·2 c.c of 0·075%	+0·2 c.c.	+0·2 c.c.	+0·2 c.c.	+0·2 c.c.	Incubation for ½ hour at 37° C.	30%
0·2 c.c of 0·1%	+0·2 c.c.	+0·2 c.c.	+0·2 c.c.	+0·2 c.c.		30%
0·2 c.c of 0·2%	+0·2 c.c.	+0·2 c.c.	+0·2 c.c.	+0·2 c.c.		30%
0·2 c.c. of 0·3%	+0·2 c.c.	+0·2 c.c.	+0·2 c.c.	+0·2 c.c.		30%

CERTAIN ASPECTS OF BLACK-WATER FEVER

It is not intended in this paper to discuss the ætiology of black-water fever, nor shall I discuss those cases in which hæmoglobinuria may occur without any administration of quinine, but the main subject of this paper is to discuss how to treat cases of malarial fever in individuals who are susceptible to attacks of hæmoglobinuria after administration of quinine. The position becomes extremely difficult, because cases of severe malignant tertian infection may come under one's treatment in which administration of quinine is followed by hæmoglobinuria which may end fatally. Such cases, though rare, still do occur. The following examples of this type of cases are given here to show how extremely difficult it would be to treat them.

Case I

The patient, aged 12, came for treatment of malignant tertian fever. He gave an account of attacks of hæmoglobinuria after administration of quinine. When I saw the case, he was suffering from intermittent fever. The spleen was slightly enlarged, blood showed a fair number of malignant tertian parasites. I put the patient on large doses of alkalies and gave him an intramuscular injection of quinine bihydrochlor of $4\frac{1}{2}$ grains on the first day and 9 grains on the second day. This was followed by high rise of temperature, hæmoglobinuria and eventually the case ended fatally.

Case II

The patient, aged 30, came for treatment with the follow-ing history. He had an attack of hæmoglobinuria seven days previously, after administration of quinine for the treat-ment of an attack of malarial fever. When he came to me, the hæmoglobinuria had stopped. He had a slightly enlarg-ed spleen and no parasites could be found in the blood. The patient was put on calcium chloride and alkalies. This treatment was continued for ten days, when his blood was examined for malarial parasites and only a few crescents were found in the blood. He had no fever during this period. I decided to give quinine with the hope of preventing a relapse and five grains of quinine hydrochlor dissolved in hydrochloric acid were administered at 11 a.m. This was followed by a rigor and rise of temperature up to 102° F at 2 p.m. Intense hæmoglobinuria started about the same time. The temperature rose up to 105° F. at 10 p.m. and the patient died at about 12 p.m., i.e., about 13 hours after administration of quinine.

Case III

This case is most interesting, as I could regulate the degree of hæmoglobinuria by varying the dose of quinine. Patient, a boy aged 6 years, son of a medical man, gave history of attacks of malarial fever followed by hæmoglobi-nuria, jaundice and high rise of temperature, each time quinine was administered to him. When the patient came under my observation, he was suffering from fever with enlargement of spleen. Blood showed the presence of malignant tertian parasites. He had no quinine for nearly a month before he came to me.

The smallest dose of a quinine compound tried by me in this case and which gave rise to hæmoglobinuria was 1/12

gr. of quinine bihydrochlor. or quinine base or euquinine given twice a day. A grain of these drugs would give rise to pain in the abdomen and the limbs, jaundice, well marked hæmoglobinuria and high fever. Administration of large doses of sodi. citr. and intravenous injection of calcium chloride ($1\frac{1}{2}$ to $2\frac{1}{2}$ c.c. of a 10 per cent. solution) brought about a slight increase of tolerance to these compounds, but the patient could not bear $2\frac{1}{2}$ grs. of euquinine given in divided doses of $\frac{1}{2}$ gr. each, in spite of 10 grains of sodi. citr. given four times a day and daily intravenous injection of calcium chloride ($2\frac{1}{2}$ c.c. of a 10 per cent solution). The symptoms that followed administration of $2\frac{1}{2}$ grains of quinine bihydro-chlor consisted of pain in the limbs and abdomen, extreme prostration, jaundice, quick pulse, high fever and hæmo-globinuria. In other words, the patient was almost abso-lutely intolerant to any form of quinine.

Before proceeding to discuss the treatment of the extremely difficult cases of the type mentioned above, I would refer here to certain conclusions based on published observations of mine on the mechanism of black-water fever and quinine hæmoglobinuria and on the hæmolytic effect of certain salts of quinine.

A.—Mechanism of Quinine Hæmoglobinuria and that of Black-water Fever

(1) No hæmolysin has been discovered in the peripheral blood during the active stage of quinine hæmoglobinuria.

(2) Patients who bear quinine well at one time, may subsequently develop tendency to quinine hæmoglobi-nuria.

(3) The mechanism of quinine hæmoglobinuria is similar to that of black-water fever in respect to the fact that in both the processes hæmolysis takes place in at least one internal organ, namely, the liver.

(4) In certain cases of black-water fever, the hæmoglobinuria is of an intermittent nature increasing during height of fever and diminishing during apyrexia.

(5) Quinine hæmoglobinuria may he manifested in individuals suffering from kala-azar and in whom there may be no connection with any recent attacks of malaria

(6) The red corpuscles of patients having a tendency to quinine hæmoglobinuria are not more vulnerable to quinine bihydrochlor than those of individuals having no tendency to' quinine hæmoglobinuria.

References

[1] Indian Journal of Medical Research, Oct., 1925 and January, 1926.
[2] Bio chemical Journal, Vol. XV, No. 4, 1921.
[3] Indian Medical Gazette, June, 1921.
[4] The Indian Journal of Medicine, Vol. X, Part 1. February, 1929.

SOME OBSERVATIONS ON THE HÆMO-LYTIC ACTION OF CERTAIN QUININE SALTS ON THE ERYTHROCYTES OF DIFFERENT INDIVIDUALS AND ON THE RESISTANCE OF NEWLY FORMED RED CORPUSCLES TO HÆMOLYSIS UNDER THE INFLUEN E OF DISTILLED WATER

I.   In, the first part of this paper are recorded the observations made by us on the hæmolysis of erythrocytes by certain salts of quinine in a series of patients taken promiscuously from the medical wards of a hospital of which one of us is in charge.   The research was conducted with a view to determine whether the salts of quinine vary in their hæmolytic action on the red corpuscles of different individuals and how far the quinine radicle or the acid portion of a quinine salt is responsible for the hæmolysis.

A.   Table showing the hæmolytic action of different salts of quinine upon the red corpuscles of different individuals

	Q. Bihydro-chlor	Q. Hydro-chlor.	Q. Bi-sulph	Q. Sulph.	Hydrochlor acid.	Sulphuric acid.
Man	0·040	0·45	0 055	0·45	0·015	0·025
,,	0·050	0 50	0·070	0·50	0·020	0·030
,,	0·055	0·45	0·070	0·45	0·020	0 030
,,	0 060	0 50	0·0775	0·50	0 025	0·030

	Q Bihydro-chlor.	Q. Hydro-chlor.	Q. Bi-sulph.	Q. Sulph.	Hydrochlor acid	Sulphuric acid.
Man	0·050	0·37	0·070	0·40	0·015	0·025
,,	0·085	0·40	0·100	0·45	0·027	0·037
,,	0·080	0·30	0·095	0·325	0·030	0·035
,,	0·055	0·40	0·070	0·45	0·020	0·030
,,	0 085	0·40	0·100	0·50	0·035	0·043
,,	0 060	0·30	0·075	0·35	˙ 0·025	0·030
,,	0·045	0·45	0 060	0·50	0·020	0·030
,,	0·035	0·45	0·045	0·50	0·015	0·020
,,	0 060	0·30	0 075	0·35	0·025	0·030
,,	0 055	0·35	0 070	0·35	0·025	0·035
,,	0·070	0·30	0·080	0·35	0·030	0·035
,,	0·080	0·35	0·095	0·40	0·035	0·040
·,	0·055	0·30	0·070	0·35	0·025	0·030
,,	0·050	0·425	0·070	0·45	0·020	0·025
,,	0·065	0·35	0·075	0·35	0·030	0·030
Fowl	0·100	0·40	0·120	0·45	0·035	0·045

We shall not enter here into the researches of previous workers on the hæmolytic action of quinine salts. The reader is referred to them for comparison of our results on those points in which work has already been done by them.

In all our observations, the experiments were made with solutions of quinine salts in normal saline.

Each observation extended from $2\frac{1}{2}$ to 3 hours, the test tubes containing the mixture of washed corpuscles and quinine solution being kept at 40°C. The corpuscles were washed with 0·85 per cent. NaCl solution.

Columns (1), (2), (3) and (4) show the strength of the solution of a quinine salt (g. per 100 cc.) in which the red corpuscles begin to hæmolyse in the course of $2\frac{1}{2}$ to 3 hours. Columns (5) and (6) show the percentage of HCl or H_2SO_4 in a solution calculated in terms of quinine bihydrochloride or quinine bisulphate in which the same amount of acid is present. Thus 0·015 per cent. hydrochloric acid means the amount of hydrochloric acid present in 100 c.c. of normal saline containing 0·015 g. of quinine bihydrochloride.

From the above the following conclusions may be drawn :

(1) Quinine bihydrochloride is the most hæmolytic of the quinine salts shown in the table.

(2) The acid salts are more hæmolytic than the corresponding neutral salt.

(3) Free hydrochloric acid or free sulphuric acid is more hæmolytic than a solution of quinine bihydrochloride or quinine bisulphate containing the same amount of hydrochloric acid or sulphuric acid respectively.

It will be seen that the presence of the quinine radicle in a quinine salt retards the hæmolytic action of the acid present in the salt.

From the above table it will be seen that the erythrocytes of different individuals vary differently in their resistance to hæmolysis under the influence of a quinine salt and it is quite possible that we might come across an individual in whom the resisting power of the red corpuscles was still lower. Black-water fever is therefore likely to occur in those individuals in whom the erythrocytes are least resistant to the action of a quinine salt either owing to disease or to a natural weakness on their part in resisting the action of the acid portion of a quinine salt.

In our investigations we have found that fowl's corpuscles are more resistant to hæmolysis under the influence of a quinine salt than the average red corpuscles of man, and therefore any conclusion drawn from observations on the haemolytic action of quinine salts in lower animals may be fallacious, if applied to the case of man.

Attempts have been made by us to determine whether the hæmolytic power of a quinine salt is diminished by the presence of glucose in its solution. One such experiment has, up to the time of writing, been made in the case of the fowl and the following results were obtained :

Commencing hæmolysis of fowl's red corpuscles was obtained with :

(1) 0˙1 % solution of quinine bihydrochloride, (2) 0˙4% solution of quinine hydrochloride, (3) 0˙12 per cent solution of quinine bisulphate all in normal saline.

(2) 0˙12% solution of quinine bihydrochloride, (2) 0˙45% solution of quinine bisulphate, (3) 0˙14 per cent solution of quinine bisulphate, all in normal saline containing 5 per cent glucose.

It is thus seen that glucose retards the hæmolytic action of salts of quinine.

II. The second part of this paper refers to a series of investigations made to determine the resisting power of the red corpuscles, present after repeated bleeding, to hæmolysis under the influence of distilled water.

In this Journal and in his *Studies in Hæmolysis* one of us described a method of determining the fragility of the red corpuscles which may be briefly described as follows :

One part of blood is treated with two parts of distilled water and the proportion of hæmoglobin in the undissolved corpuscles to that of the total volume of blood taken is estimated. This gives a factor which is fairly constant under normal conditions and may be termed the relative hæmoglobin-value of the resistant corpuscles. In the case of the fowl, this factor was estimated to be 16˙85 as an average of a large number of observations.

To obtain absolutely identical conditions, the blood of the fowl in each case was washed three times with 0˙85 NaCl per cent solution, so that the osmotic pressure of the suspending fluid of the corpuscles in each case was exactly the same.

The corpuscles after repeated bleeding were obtained in the following way :—the fowls were repeatedly bled from their veins in the wings till the total quantity of blood removed amounted to 30 to 50 c.c. The hæmoglobin-value

of the resistant corpuscles after repeated bleeding was estimated seven days after the last bleeding and compared with their hæmoglobin-value before bleeding.

The following experiments were performed :

I. Weight of fowl—14$\frac{3}{4}$ oz. Total amount of blood removed after bleeding for four times = 27 c.c. Hæmoglobin-value of resistant corpuscles before bleeding = 17, after bleeding = 35·5.

II. Weight of fowl—3 lbs. 5$\frac{3}{4}$ oz. Total volume of blood removed after repeated bleeding for five times = 44 c.c. Hæmoglobin-value of the resistant corpuscles before bleeding = 15, after bleeding = 37.

III. Weight of fowl—3 lbs. 12 oz. Total volume of blood removed after repeated bleeding for four times = 34 c.c. Hæmoglobin-value of the resistant corpuscles before bleeding = 17, after bleeding = 32·5.

IV. Weight of fowl—3 lbs. 14 oz. Total volume of blood removed after repeated bleeding for six times = 54 c.c. Hæmoglobin-value of the resistant corpuscles before bleeding = 11, after bleeding = 20·5.

V. Weight of fowl—3 lbs. Total volume of blood removed after repeated bleeding = 41 c.c. Hæmoglobin-value of the resistant corpuscles before bleeding = 10, after bleeding = 17·7.

It will be seen that in all the above cases, the resistant corpuscles were increased after repeated bleeding, thus proving that red corpuscles obtained under these conditions are more resistant to hæmolysis than under normal conditions.

STUDIES IN QUINOLINE COMPOUNDS

Part 1

The object of this paper is to study a series of quinoline compounds with the view of discovering those that are likely to possess anti-protozoic and therapeutic properties.

Of the compounds described here, the first series contains a vinyl grouping. The presence of vinyl grouping in these compounds is comparable to the same group being present in quinine and allied compounds having therapeutic properties. These compounds possess certain definite properties, which are absent in the second series They are generally yellow in colour and exhibit a blue fluorescence in dilute alcoholic solution. They are extremely insoluble in water, but dissolve fairly in alcohol, acetone and ether. They give a dark red colouration with acids. Their salts are hydrolysed in excess of water.

These compounds have been prepared from quinaldine and its derivatives by condensing them with p-dimethyl-aminobenzaldehyde. The reactivity of the a-methyl group in quinaldine has been taken advantage of in its condensation with this aldehyde. In the preparation of these compounds we have not taken the help of any condensing agent as simple heating of the reactants brings about their combination.

The second series of compounds have been obtained from the various aminoquinolines by condensing them with chloracetamide. These compounds are not so much insoluble in water as compounds of the first series. They exhibit no

fluorescence. Their salts are not hydrolysed by excess of water and they are generally colourless. Acids dissolve them without giving rise to any colour.

EXPERIMENTAL

FIRST SERIES

2-p-Dimethyl-aminostyryl-quinoline
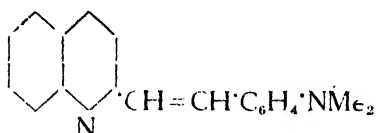
Quinaldine (2g.) and *p*-dimethylaminobenzaldehyde (2g.) are heated carefully in a dry test tube to gentle boiling for about 15 minutes over a small flame. The mixture is then dissolved in boiling alcohol and filtered. The filtrate on cooling deposits the above compound. It is then collected and dried (*vide* Neolting and Witter, *Ber.*, 1906, **39**, 2479); m. p. 175°. (Found: N, 10·5. $C_{19}H_{18}N_2$ requires N, 10·2 per cent.).

6-Methyl-2-p-dimethyl-aminostyryl-quinoline
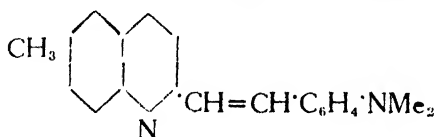
6-Methylquinaldine (1 g.) is mixed in a dry test tube with *p*-dimethylaminobenzaldehyde (1g.) and the mixture heated carefully to gentle boiling over a small flame for about 15 minutes. The substance is purified and dried in the same way as in the previous case. It melted at 199°. (Found: N, 9·9. $C_{20}H_{20}N_2$ requires N, 9·7 per cent.).

6-Oxy-2-p-dimethyl-aminostyryl-quinoline

6-Oxyquinaldine (2g.) and *p*-dimethylaminobenzaldehyde (2g.) are mixed together and heated carefully to gentle

boiling over a small flame for about 10 minutes. Acetone is then added in excess to the product. The hydroxy-derivative remains undissolved. It is separated and dried; m.p. above 240°. (Found: N, 9·89. $C_{19}H_{18}QN_2$ requires N, 9·65 per cent.).

6-*Ethoxy-2-p-dimethyl-aminostyryl-quinoline*

6-Ethoxyquinaldine (2g.) and *p*-dimethylaminobenzaldehyde (2g.) are heated carefully to gentle boiling over a small flame for 10 minutes. The product is poured into water. The solid thus separated is then purified by recrystallisation from ether; m. p. 212°. (Found: N, 9·15. $C_{21}H_{22}ON_2$ requires N, 8·81 per cent.).

6-*Methoxy-2-p-dimethyl-aminostyryl-quinoline*

6-Methoxyquinaldine (2g.) and *p*-dimethylaminobenzaldehyde (2g.) are condensed by heating for 15 minutes. The product is then dissolved in alcohol. Water precipitates the base from the alcoholic solution. It is then extracted with ether. The ethereal solution is then dried over fused calcium chloride and the ether removed. The residual solid thus obtained is then dried in vacuum; m.p. 202°. (Found: N, 9·45. $C_{20}H_{20}ON_2$ requires N, 9·2 per cent.).

SECOND SERIES

Quinoline-6-aminoacetamide, $NH_2\cdot CO\cdot CH_2\cdot NH$
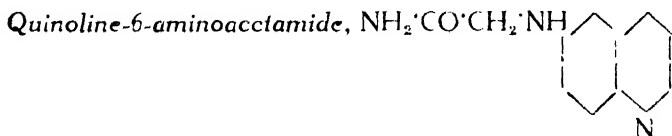
6-Aminoquinoline hydrochloride (4g.) is dissolved in 20 c.c. of water and chloracetamide (2·5g.) added to it. The whole is then boiled for about 3 hours. It is then filtered, and the filtrate cooled with ice and made alkaline with a saturated solution of sodium carbonate, when the

required compound separates out. It is filtered, recrystallised from alcohol and dried on a porous plate; m.p. 197°. (Found : N, 21·3. $C_{11}H_{11}ON_3$ requires N, 20·9 per cent.).

Quinoline-8-aminoacetamide

This compound is prepared by boiling a solution of 8-aminoquinoline hydrochloride and chloracetamide in water in equimolecular proportions for 3 hours. The condensation product is then separated by adding sodium carbonate and then purified by recrystallising from alcohol; m.p. 183°. (Found : N, 21·25. $C_{11}H_{11}ON_3$ requires N, 20·9 per cent.).

6-Ethoxy-quinoline-8-aminoacetamide

6-Ethoxy-8-aminoquinoline hydrochloride and chloracetamide are dissolved in water in equimolecular proportions and the aqueous solution is boiled for 3 hours. The final product is isolated and purified as above ; m.p. 235°. (Found : N, 16·88. $C_{13}H_{15}O_2N_3$ requires N, 17·1 per cent.).

6-Methoxy-quinoline-8-aminoacetamide

Equimolecular proportions of 6-methoxy-8-aminoquinoline hydrochloride and chloracetamide are dissolved in water and the solution boiled for 3 hours. The product is separated and purified in the usual way; m.p. 226°. (Found : N, 17·89. $C_{12}H_{13}O_2N_3$ requires N, 18·2 per cent.).

STUDIES IN QUINOLINE·COMPOUNDS

Part II

SOME DERIVATIVES OF 4-PHENYL-2-METHYL-QUINOLINE

The enhanced toxicity of 4-phenyl-2-methylquinoline
towards certain kinds of protozoa when compared with that
of quinoline and quinaldine induced us to synthesise a few
derivatives of this important compound. Up till now only
very few compounds of this series have been synthesised.
In the synthesis of these compounds we have taken the help
of the reaction of Doebner and v. Miller modified by
Beyer (*J. p. Chem.*, 1886, *ii*, 33, 393). In this way we
have obtained

(*i*) 6-nitro-4-phenyl-2-methylquinoline,

(*ii*) 8-nitro-4-phenyl-2-methylquinoline, and a few
derivatives of them.

By heating *p*- and *o*-nitranilines respectively with
benzoylacetone, little or no condensation takes place.
Similarly on heating *p*-nitraniline hydrochloride with a
mixture of paraldehyde and acetophenone, already saturated
with dry hydrochloric acid gas, very little or scarcely any,
condensation takes place. But by certain modifications of
the latter method described below we have ultimately
succeeded in getting the above condensation products,
and the increased basic properties of these substances
enabled us in separating them from their parent substances.

These nitro-compounds are of a very pale yellowish green
colour. They readily dissolve in dilute acids. Stannous

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chloride and hydrochloric acid easily reduce them to the corresponding amines which can be separated through their finely crystalline stannichlorides. They also react, rather easily, with p-dimethylaminobenzaldehyde to give the corresponding styryl compounds.

EXPERIMENTAL

6-Nitro-4-phenyl-2-methylquinoline

A mixture of acetophenone (50 c.c.) and paraldehyde (30 c.c.) is cooled in ice and saturated with dry hydrochloric acid gas. This mixture is then allowed to stand for 48 hours. It is then again saturated in cold with dry hydrochloric acid gas and allowed to stand for 24 hours more. This is now slowly added to an intimate mixture of p-nitraniline (58 g.) and concentrated hydrochloric acid (90 c.c.) and the whole heated for 5 to 6 hours on a water-bath with occasional shaking. It is then largely diluted with water and the supernatant liquid carefully filtered through a coarse filter paper. The clear filtrate is then cooled by adding ice. A strong aqueous solution of sodium hydroxide is then cautiously added to precipitate nearly the whole of the unreacted p-nitraniline. It is then filtered and the filtrate supersaturated with alkali. The required nitrophenyl-2-methylquinoline thus precipitated, is separated by filtration, washed with water and dried on a porous plate. Under this condition it has a fine greenish colour. It can be further purified by recrystallisation from a mixture of acetone and ether.

The pure compound, m.p. 141°, possesses a yellowish-green colour. It gives precipitates with potassium dichromate, phospho-molybdic acid and picric acid and dissolves very easily in alcohol and acetone and moderately in ether and in boiling water. The solution of the compound in dilute mineral acids possesses an orange-yellow colour. (Found : N, 11·1. $C_{16}H_{12}O_2N_2$ requires N, 10·6 per cent.).

6-Amino-4-phenyl-2-methylquinoline

The above nitro-compound can be conveniently reduced as follows : To a mixture of stannous chloride (30 g.) and concentrated hydrochloric acid (50 c.c) the nitro-compound (6·5 g.) is gradually added under constant shaking. If the mixture gets very hot, it must be cooled under the tap. On cooling the fine crystalline stannichloride of the amine crystallises out. It is filtered, washed with concentrated hydrochloric acid and dissolved in about 200 c.c. of boiling water. The tin is precipitated as sulphide and removed by filtration. The filtrate when made alkaline with dilute sodium hydroxide precipitates the amine as fine needles. It is then recrystallised from ether; m.p. 188°. It can be diazotised and coupled with β-naphthol. The solution of the substance in acetone possesses a beautiful violet-blue fluorescence. (Found : N, 12·3. $C_{16}H_{14}N_2$ requires N, 12·0 per cent.).

6-Nitro-4-phenyl-2-p-dimethyl-aminostyryl-quinoline

This compound can be easily prepared by heating to gentle boiling over a small flame, a mixture of 6-nitro-4-phenyl-2-methylquinoline and p-dimethylaminobenzaldehyde in equimolecular proportions for about 10 to 15 minutes. The product is then poured into water. The solid thus separated is then purified by recrystallisation from acetone. It has a brownish-red colour and melts at 64°. (Found : N, 10·8. $C_{25}H_{21}O_2N_3$ requires N, 10·6 per cent.).

8-Nitro-4-phenyl-2-methylquinoline

A mixture of acetophenone (50 g.) and paraldehyde (30 g.) is saturated with dry hydrochloric acid gas as described before. This is then added to a mixture of o-nitraniline (58 g.) and concentrated hydrochloric acid (90 g.) and

the whole heated on a water-bath for 7 to 8 hours. The product is then largely diluted (to about 2 litres) and, after filtration through a coarse filter paper, the filtrate is made strongly alkaline with sodium hydroxide. The o-nitraniline remains in solution while the 8-nitro-4-phenyl-2-methylquinoline precipitates out as a greenish-black pasty mass. It is then dried on a porous plate and recrystallised from acetone. The compound looks pale yellowish-green; m. p. 94°. In its other behaviour it resembles the corresponding 6-nitro-compound. (Found : N, 10·9. $C_{16}H_{12}O_2N_2$ requires N, 10·6 per cent.).

8-Amino-4-phenyl-2-methylquinoline

The above nitro-compound can also be reduced very easily by stannous chloride just as in the previous case. The filtrate, after the separation of tin sulphide, is evaporated on a water-bath when the amine hydrochloride remains behind. It is very difficult to get the free amine from this hydrochloride. The hydrochloride possesses an yellow colour and begins to decompose above 210°. It dissolves very easily in water to give an orange-yellow solution. (Found : N, 10·7. $C_{16}H_{15}N_2Cl$ requires N, 10·3 per cent.).

8-Nitro-4-phenyl-2-p-dimethyl-aminostyryl-quinoline

The preparation and purification of this compound are exactly similar to those of the corresponding 6-nitro-compound, 8-nitro-4-phenyl-2-methylquinoline being substituted for the 6-nitro-4-phenyl-2-methylquinoline. It melts at 129°. (Found : N, 10·9. $C_{25}H_{21}O_2N_3$ requires N, 10·6 per cent.).

STUDIES IN QUINOLINE COMPOUNDS

Part III

The fact that certain derivatives of quinoline (" plasmo-chin ") and certain arsenicals possess antimalarial properties, led us to synthesise some arsenic derivatives of quinoline. The first idea that would strike a worker in this direction is to attempt to introduce arsenic directly into the quinoline nucleus. Up till now, such a method has not been found feasible except in the case of quinaldine (Frankel and Lowy, *Ber.*, 1913, 46, 2549). The compound has been obtained indirectly by the application of Döbner-Miller's reaction to *p*-aminophenylarsonic acid.

With the view of introducing arsenic directly into deri-vatives of quinoline we first tried the method of Bart (German Patent, 250264) as well as Mouneyrat's modification of it (British Patent, 142947). In all our attempts we obtained dyes and reduction products only. No compound containing arsenic was isolated during a prolonged investigation in this direction.

We next tried the mercuric acetate method, but here we found that during the course of the reaction the mercury was almost quantitatively reduced to the metallic state.

Subsequently we tried to condense aminoquinolines with aromatic arsenicals. With this object, chloracetyl-*p*-arsani-lic acid was condensed with various aminoquinolines, follow-ing the method of Jacobs and Heidelberger (*J. Amer. Chem.*

Soc., 1919, 41, 1810). The compounds enumerated below were thereby obtained :—

(1) Quinoline-8-aminoacetyl-*p*-arsanilic acid.

(2) 6-Methoxyquinoline-5-aminoacetyl-*p*-arsanilic acid.

(3) Quinoline-6-aminoacetyl-*p*-arsanilic acid.

(4) 2-Methylquinoline-6-aminoacetyl-*p*-arsanilic acid.

These compounds are generally yellow in colour. They are insoluble in water, very sparingly soluble in common organic solvents such as alcohol, ether, acetone, etc. They however, dissolve very easily in aqueous caustic alkalis. From the alkaline solutions, acids precipitate the original compound. Continued boiling with dilute alkali causes hydrolysis. They are sparingly soluble in dilute mineral acids.

On treating with an excess of sodium nitrite solution in 50 per cent acetic acid beautiful crystalline nitroso-derivatives are obtained.

For the sake of examining the therapeutic properties we also prepared arsanilic acid salts of 8- and 6-aminoquino-lines.

EXPERIMENTAL

Quinoline-8-aminoacetyl-p-arsanilic acid

$$NH.CH_2.CO.NH-\left\langle\underline{\quad}\right\rangle-AsO(OH)_2$$
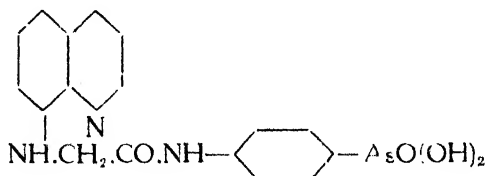
Chloracetylarsanilic acid (2·26 g.), prepared according to Jacob and Heidelberger's method (*loc. cit.*), is dissolved in *N*-sodium hydroxide solution (7·5 c.c.) and to it is added 8-aminoquinoline (1·6 g. in 7·5 c.c. alcohol). This mixture

is then boiled on a water bath for $\frac{1}{2}$ hour. The solid which separates out is collected, washed with very dilute hydrochloric acid and then with water. It is then dried in vacuum. The compound has a deep yellow colour and dissolves very easily in aqueous alkalis, diethylamine and also in concentrated hydrochloric acid. It is very difficultly soluble in all other solvents. M.P. 171° (decomp.). (Found: N, 10·2; As, 18·2. $C_{17}H_{16}O_4N_3As$ requires N, 10·5; As, 18·7 per cent.).

The *sodium salt* of the above compound is prepared by dissolving the moist acid in the smallest quantity of N-sodium hydroxide and then precipitating with an excess of alcohol. It is very easily soluble in water.

The *nitroso-derivative* is prepared by suspending 1 g. of the acid in 10 c.c. of 50 per cent acetic acid and then treating with a solution of 1 g. of sodium nitrite. On scratching the solution, the nitroso-compound separates. It has got a greenish yellow colour and decomposes with effervescence at about 182°. (Found: N, 12·8. $C_{17}H_{15}O_5N_4As$ requires N, 13·00 per cent.).

6-Methoxyquinoline-5-aminoacetyl-p-arsanilic acid

The compound is prepared from 6-methoxy-5-aminoquinoline and chloracetyl-p-arsanilic acid, the procedure being the same as in the case of the above compound. It is of a dirty grey colour and easily soluble in alkalis but sparingly in all other solvents. It does not melt even at 240°. (Found: N, 9·5. $C_{18}H_{18}O_5N_3As$ requires N, 9·7 per cent.).

The *sodium salt*, prepared as indicated above, is very easily soluble in water.

The *nitroso-derivative* is an almost white substance and does not melt at 240°. (Found: N, 12·1. $C_{18}H_{17}O_6N_4As$ requires N, 12·2 per cent.).

Quinoline-6-aminoacetyl-p-arsanilic acid

This compound is prepared from 6-aminoquinoline and chloracetylarsanilic acid in the usual manner.

It is a greenish-yellow powder, easily soluble in caustic alkalis, but practically insoluble in all organic solvents. It does not melt at 240°. (Found: N, $10\cdot3$ $C_{17}H_{16}O_4N_3As$ requires N, $10\cdot5$ per cent.). The *sodium salt* is prepared by dissolving the free acid in the smallest amount of dilute sodium hydroxide and then precipitating with alcohol. It is very easily soluble in water.

The *nitroso-derivative* melts at above 240°. (Found: N, $12\cdot7$. $C_{17}H_{15}O_5N_4As$ requires N, $13\cdot0$ per cent.).

2-Methylquinoline-6-aminoacetyl-p-arsanilic acid

It is obtained from 2-methyl-6-aminoquinoline and chloracetyl-p-arsanilic acid. In its properties it resembles the other compounds of the series. It does not soften below 240°. (Found: N, $9\cdot7$. $C_{18}H_{18}O_4N_3As$ requires N, $10\cdot1$ per cent.). The *sodium salt* prepared as usual is very easily soluble in water.

8-*Aminoquinoline-p-arsanilate.*—This salt is prepared by mixing aqueous solutions of equimolecular quantities of 8-aminoquinoline hydrochloride and atoxyl. It separates out on scratching for some time. It is somewhat soluble in water. The compound decomposes after a few days. It begins to soften from 210° but does not melt clearly at 240°.

6-*Aminoquinoline-p-arsanilate.*—This compound is similarly prepared from 6-aminoquinoline hydrochloride and atoxyl. It does not melt at 240°.

STUDIES IN QUINOLINE COMPOUNDS

Part IV

This paper deals with certain new quinoline derivatives which, from theoretical considerations, may be of therapeutic interest.
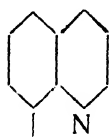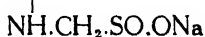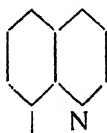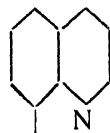
NH.CH$_2$.SO$_2$.ONa	NH.CH$_2$.SO.ONa	NH·CO·NH$_2$
(I)	(II)	(III)

The following are the series of compounds described in the present paper :

1. Sodium 8-aminoquinoline-*N*-methylenesulphonate (I).
2. Sodium 6-aminoquinoline-*N*-methylenesulphonate.
3. Sodium 6-methoxy-8-aminoquinoline - *N* - methylene-sulphonate.
4. Sodium 8-aminoquinoline-*N*-methylenesulphinate (II).
5. Sodium 6-aminoquinoline-*N*-methylenesulphinate.
6. Sodium 6-methoxy - 8- aminoquinoline- *N*-methylene-sulphinate.
7. 8-Carbamidoquinoline (III).
8. 6-Carbamidoquinoline.
9. 6-Methoxy-8-carbamidoquinoline.

These have been prepared by the replacement of one hydrogen atom of the amino-group in 6-aminoquinoline,

8-aminoquinoline and 6-methoxy-8-aminoquinoline by (*i*) $CH_2 \cdot SO_3Na$ (*ii*) $CH_2 \cdot SO_2Na$ and (*iii*) $CO \cdot NH_2$ groups.   In the last instance compounds having $\cdot NH \cdot CO$. group have been obtained which play an important part in therapeutic action in compounds such as tryparsamide, urea stibamine, antimony analogue of tryparsamide, Bayer 205, Fourneau 309, etc.

EXPERIMENTAL

Sodium   8-aminoquinoline-N-*methylenesulphonate.*—A solution of 8-aminoquinoline hydrochloride (2 g.) in 5 c.c. of water, containing alcohol (2 c.c.) is treated successively with formaldehyde (1·2 c.c. of 30%) and sodium bisulphite (2 g.) freshly dissolved in water (5 c.c.) and the whole warmed on a water-bath for $\frac{1}{2}$ to 1 hour. The precipitated base gradually dissolves when a reddish solution is obtained. It is then filtered and the clear filtrate concentrated on a water-bath and then precipitated by means of absolute alcohol. It crystallises in long yellow needles ; does not melt but dissolves readily in water to a neutral solution. (Found : N, 12·0. $C_{10}H_9O_6N_2Na$ requires N, 12·3 per cent.)

Sodium   6-aminoquinoline-N-*methylenesulphonate.*— 6-Aminoquinoline hydrochloride (1·5 g.) is treated similarly with 1 c.c. of formaldehyde and sodium bisulphite (1·5 g.) in aqueous solution. The product is obtained as reddish-brown needles after precipitation with absolute alcohol. The compound does not melt. (Found : N, 12·1. $C_{10}H_9O_3N_2Na$ requires N, 12·3 per cent.)

Sodium 6-methoxy-8-aminoquinoline-N-*methylenesulpho-nate.* —6-Methoxy-8-aminoquinoline hydrochloride when treated as above gives the sodium salt of 6-methoxy-β-aminoquinoline-*N*-methylenesulphonic acid. The former (1 g.) is dissolved in 5 c.c. of water and the solution is then warmed for some time with 1 c.c. of formaldehyde solution and 3 c.c. of 40 per cent. sodium bisulphite solution. The

solution after filtration yields the final product by preci
pitating with alcohol or acetone. It is a reddish-yellow
compound, crystallising in needles which do not melt.
(Found : N, 10·7. $C_{11}H_{11}O_4N_2Na$ requires N, 10·85 per cent.)

Sodium 8-aminoquinoline-N-methylenesulphinate. to 8-
Aminoquinoline dissolved in 10 c.c. of water is treated with
an excess of sodium formaldehyde sulphoxylate in concentrat-
ed aqueous solution and warmed on a water-bath for ½ to 1
hour. The solution is filtered and the clear filtrate pre-
cipitated by acetone or alcohol when yellow needles are
obtained. The needles do not melt below 300° but dissolve
readily in water. (Found : N, 13·1. $C_{10}H_9O_2N_2Na$ requires
N, 13·2 per cent.)

Sodium 6-aminoquinoline-N-methylenesulphinate.—The
6-aminoquinoline derivative is obtained in a similar way by
the action of an excess of sodium formaldehyde sulphoxylate
preferably in presence of alcohol (ethyl or methyl). It is a
reddish-yellow compound which shows no melting point.
(Found : N, 13·0. $C_{10}H_9O_2N_2Na$ requires N, 13·2 per cent.)

Sodium 6-methoxy-8-aminoquinoline-N-methylenesulphi-
nate.—6-Methoxy-8-aminoquinoline hydrochloride (1 g.) is
dissolved in 3 c.c. of water and 1 c.c. of alcohol. Approxi-
mately 2 g. of sodium formaldehyde sulphoxylate in
concentrated aqueous solution are added and the mixture
warmed for some time. The solution after filtration and
precipitation with alcohol gives the final product in a
crystalline form. It does not melt below 300°. (Found : N,
11·3. $C_{11}H_{11}O_3N_2Na$ requires N, 11·54 per cent.)

8-Carbamidoquinoline or Quinoline-8-carbamide.—This
compound is obtained by heating 8-aminoquinoline and
carbamide in equimolecular proportions in an oil-bath at 170°
for about an hour. It is then treated with water and filtered.
The precipitate is recrystallised from absolute alcohol. At
the beginning we expected that we would get from this
reaction a compound of the type of diquinolylcarbamide but

we found after analysis that the compound was a carbamido-derivative. This compound is identical with the one obtained from 8-aminoquinoline hydrochloride and potassium cyanate in the usual way. It crystallises in brownish-white microscopic needles, easily soluble in dilute acids, insoluble in ether, acetone or water. It melts with slight decomposition at 206°. (Found : N, 22·2. $C_{10}^4H_9ON_3$ requires N, 22·4 per cent.)

6-*Carbamidoquinoline*.—It is prepared similarly as above, from 6-aminoquinoline and carbamide. It can also be obtained from 6-aminoquinoline hydrochloride and potassium cyanate. It melts at 208°, and is easily soluble in alcohol, moderately so in acetone, and insoluble in ether or water. (Found : N, 22·1. $C_{10}H_9ON_3$ requres N, 22·4 per cent.)

6-*Methoxy*-8-*carbamidoquinoline* was obtained as above from either potassium cyanate and 6-methoxy-8-aminoquinoline hydrochloride or 6-methoxy-8-aminoquinoline and carbamide. It melts at 218°. (Found : N, 19·0. $C_{11}H_{11}O_2N_3$ requires N, 19·3 per cent.)

Observations on toxicity, pharmacological action and therapeutic properties of the above compounds are in progress.

STUDIES IN QUINOLINE COMPOUNDS
Part V

In our previous communications we prepared the simple substitution products of 8-aminoquinolines. The present paper deals with certain aminoisopropyl derivatives of 8-aminoquinolines. The characteristic feature of these compounds is that there is an assymetric carbon atom in the side-chain which is also a speciality of plasmochin. In short, these compounds can be looked upon as the lower homologues of plasmochin which, as it is understood, is an aminoisopentyl derivative of 8-aminoquinoline.

In the successive condensation of β-bromopropylphthalimide which was obtained in good yield by a modification of Seitz's method (*Ber.*, 1891, 24, 2624), with 8-aminoquinoline or its derivatives, we have followed the method of Baldwin (*J. Chem. Soc.*, 1929, p. 2962). In this case the time for condensing the β-bromopropylphthalimide was considerable, in some cases nearly 24 hours being needed for the completion of the reaction. The successive action of hydrazine in boiling alcoholic solution and hydrochloric acid on the resulting 8-β-phthalimidoisopropylaminoquinolines, effected, as was found by Baldwin, a smooth hydrolysis and the dihydrochlorides of compounds of the nature of 8-β-aminoisopropylaminoquinoline were easily isolated. In this way we have prepared the following compounds:

(1) 8-β-Aminoisopropylaminoquinoline.
(2) 8-β-Aminoisopropylamino-6-methylquinoline.
(3) 8-β-Aminoisopropylamino-6-methoxyquinoline.
(4) 8-β-Aminoisopropylamino-6-ethoxyquinoline.

The free bases are not usually crystallisable and therefore we have not isolated them. The bihydrochlorides are solids which can be easily crystallised from absolute alcohol. These dissolve very easily in water giving rise to a faintly acidic solution. The alkylation of these compounds is not very difficult, but the products cannot be obtained in a very pure state.

For the sake of comparison we have also prepared the (1) 8-β-aminoethylaminoquinoline, (2) 8-β-aminopropyl-aminoquinoline.

We would point out here that the condensation of β-bromopropylphthalimide can only be effected with 8-aminoquinoline and its derivatives, whereas the isomeric 6-, 7-, or 5-aminoquinolines cannot be made to undergo this condensation.

EXPERIMENTAL

8-β-Aminoisopropylaminoquinoline.

The starting materials are β-bromopropylphthalimide and 8-aminoquinoline, of which the former is prepared by heating allylphthalimide (6 g.) with fuming hydrobromic acid (d 1·78, 10 c.c.) for about 10 minutes on a boiling water-bath and then pouring into water. The solid thus separated is then crystallised from dilute alcohol, m.p. 150°.
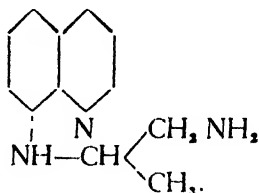
β-Bromopropylphthalimide (5 g.) is intimately mixed with 8-aminoquinoline (3 g.) and heated on an oil-bath at 125-35° for about 24 hours, when the separation of the solid

hydrobromide is nearly complete. The nearly solid mixture is then stirred up with absolute alcohol and filtered. The precipitate is washed thoroughly with alcohol and dried in vacuum, yield 4 g.

The above hydrobromide (4 g.) is suspended in absolute alcohol (25 c.c.) and 1·5 c.c. (2·5 mols.) of hydrazine hydrate added into it. The mixture is heated on the water-bath for 1½ to 2 hours. The alcohol is then driven off and the white spongy residue is mixed with an excess of 2N-hydrochloric acid and heated on a boiling water-bath for 15 minutes. The precipitated phthalylhydrazide is filtered off and the filtrate basified. The base is taken up with chloroform. On passing dry hydrogen chloride into the dry chloroform solution, the bihydrochloride is precipitated, and is recrystallised from absolute alcohol. It is a brownish yellow solid, easily soluble in water, m.p. 235°. The solution is only faintly acidic. (Found : N, 15·5. $C_{12}H_{17}N_3Cl_2$ requires N, 15·3 per cent.)

8-β-*Aminoisopropylamino*-6-*methylquinoline*.—The preparation of this compound is almost the same as the previous one. The separation of the intermediate hydrobromide is nearly complete within 12 hours. The bihydrochloride is a brownish yellow solid melting at 255°. It is very easily soluble in water, moderately soluble in absolute alcohol from which it is recrystallised. (Found : N, 14·3. $C_{13}H_{19}N_3Cl_2$ requires N, 14·6 per cent.)

8-β-*Aminoisopropylamino*-6-*methoxyquinoline* is prepared from β-bromopropylphthalimide (4 g.) and 6-methoxy-8-aminoquinoline (2·5 g.). It is a yellow solid melting at 221°. (Found : N, 13·7. $C_{13}H_{19}ON_3Cl_2$ requires N, 13·8 per cent.)

8-β-*Aminoisopropylamino*-6-*ethoxyquinoline* melts at 231° (Found : N, 13·0. $C_{14}H_{21}ON_3Cl_2$ requires N, 13·2 per cent.)

8-β-Aminoethylaminoquinoline.

NH·CH$_2$·CH$_2$·NH$_2$
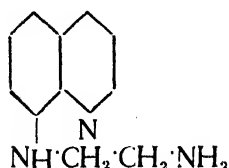
It is prepared from 8-aminoquinoline and β-bromoethyl-phthalimide. It melts at 241°. (Found : N, 16·4. C$_{11}$H$_{15}$N$_3$Cl$_2$ requires N, 16·3 per cent.)

8-γ-Aminopropylaminoquinoline.—It is obtained as above from 8-aminoquinoline and γ-bromopropylphthalimide. It melts at 247°. (Found : N, 15·5. C$_{12}$H$_{17}$N$_3$Cl$_2$ requires N, 15·3 per cent.)

STUDIES IN QUINOLINE COMPOUNDS
Part VI

The first series of compounds investigated in this paper are alkylaminoquinoline derivatives which bear close relationship to plasmoquine type of compounds. The general contour is very similar ; while position 8 is occupied by amino group, position 6 is occupied by a methoxy-, ethoxy—or chloro-group. Further attachment of an alkyl-amino group to the former makes the general structure akin to that usually ascribed to plasmoquine, the constitution of which, however, is not definitely known. The object of preparing these compounds is, therefore, to approach the constitution of plasmoquine in a systematic way. The similarity of the compounds investigated with plasmoquine will be apparent from the following structures :—

Plasmoquine, probable structure)
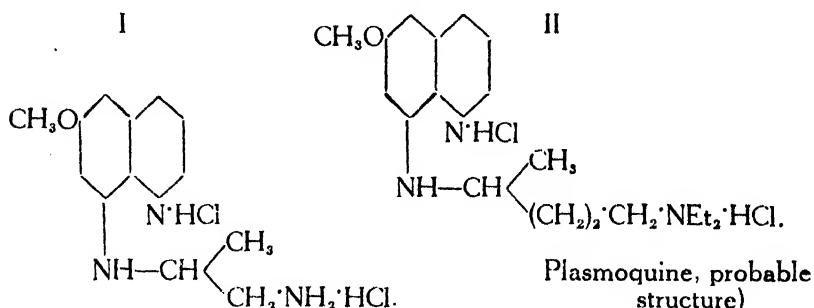
It will be noticed that the alkylaminoquinolines prepared by us contain asymmetric carbon atoms which are also present in plasmoquine and quinine.

The second series of compounds are characterised by having unsaturated linkages (attached to the amino group in position 8), which are also present in quinine though not in plasmoquine.

In all cases the hydrochlorides have been prepared as these are more easily ·crystallised than the corresponding bases; moreover, the bases are insoluble in water while the hydrochlorides are readily soluble and are so well adapted for pharmacological experiments.

EXPERIMENTAL

First Series

6-*Methoxy*-8-*β-aminoisopropyl-aminoquinoline dihydro-chloride.*
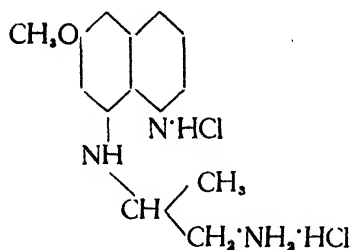
The preparation of this compound is given here in greater detail than in our previous paper (*J. Indian Chem. Soc.*, 1931, 8, 571). The starting materials for the synthesis of this compound are 6-methoxy-8-aminoquinoline and β-bromo*iso*propylphthalimide. The former is prepared by nitrating acetyl-*p*-anisidine and subsequent hydrolysis of the acetyl group.

The amino compound thus obtained is then subjected to Skraup's reaction which gives 6-methoxy-8-aminoquinoline (British Pat., 267, 169). This is then condensed with β-bromo*iso*propylphthalimide. This latter is obtained by condensing phthalic anhydride with allylamine and then warming the resulting allylphthalimide with fuming HBr or alternately

by condensing allyliodide with potassium phthalimide and then proceeding as above (*cf.* Seitz, *Ber.*, 1891, 24, 2624).

6-methoxy-8-aminoquinoline (1 g.) and bromoisopropyl-phthalimide (2 g.) are boiled together in an oil-bath at 130° for about 6 hours, at the end of which the mass solidifies. The product is then treated with alcohol which dissolves the excess of bromopropylphthalimide and aminoquinoline, while the hydrobromide of phthalimido*iso*propylaminomethoxy-quinoline remains insoluble. It is then filtered, washed with alcohol and dried. As it is difficult to hydrolyse it, its hydrazine condensation product is prepared, which can be easily hydrolysed (*cf.* Ing and Muske, *J. Chem. Soc.*, 1926, p. 2348).

The hydrobromide (5 g.) is treated with alcohol (3 c.c.) and hydrazine hydrate (0·1 c.c.) next added. The whole is refluxed for 1½ hours and alcohol is distilled off while a white spongy mass remains. This latter is then hydrolysed by warming for about 15 minutes on a water-bath with excess of dilute hydrochloric acid. The mixture is filtered, the filtrate is made alkaline and the precipitated base is extracted with chloroform. The chloroform solution is then treated with HCl gas, when the dihydrochloride is obtained. This is purified from alcohol in yellow crystalline form melting at 218-20°. It readily dissolves in water to a clear yellow solution. (Found : N, 13·70 ; Cl, 23·30. $C_{13}H_{19}ON_8Cl_2$ requires N, 13·81 ; Cl, 23·35 per cent.)

*2-Methyl-6- methoxy -8- β-amino*iso*propyl- aminoquinoline dihydrochloride.*
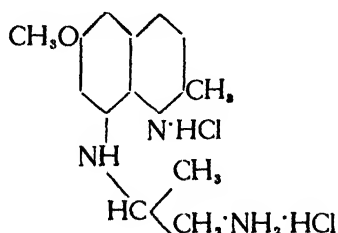
It is obtained similarly from 2-methyl-6-methoxy-8-amino-quinoline which is prepared by applying Doebner and Miller's reaction to 4-methoxy-2-nitraniline and subsequent reduction. It is a yellow crystalline compound which melts at 260°. (Found : N, 13·30; Cl, 22·20. $C_{14}H_{21}ON_3Cl_2$ requires N, 13·34; Cl, 22·33 per cent.)

2-Methyl-6-ethoxy-8-β-aminoisopropyl-aminoquinoline dihydrochloride.—It is obtained from 2-methyl-6-ethoxy-8-aminoquinoline which results from Doebner and Miller's reaction on the corresponding nitraniline and subsequent reduction. It melts at 270°. (Found : N, 12·70; Cl, 21·42. $C_{15}H_{23}ON_3Cl_2$ requires N, 12·65; Cl, 21·38 per cent.)

6-Chloro-8-β-aminopropyl-aminoquinoline dihydrochloride.—It is obtained as before from 6-chloro-8-aminoquinoline. It is a yellow crystalline compound melting at 212°. (Found : N, 13·50; Cl, 34·41. $C_{12}H_{16}N_3Cl_3$ requires N, 13·61 ; Cl, 34·52 per cent.)

2-Methyl-6-chloro-8-β-aminoisopropyl-aminoquinoline dihydrochloride.—It is obtained as above from 2-methyl-6-chloro-8-aminoquinoline. It melts at 255°. (Found : N, 13·1 ; Cl, 33·1. $C_{13}H_{18}N_3Cl_3$ requires N, 13·02; Cl, 33·02 per cent.)

2-Methyl-8-β-aminoisopropyl-aminoquinoline dihydrochloride.—It is prepared similarly from 2-methyl-8-aminoquinoline. It melts at 275-80°. (Found : N, 14·50; Cl, 24·42. $C_{13}H_{19}N_3Cl_2$ requires N, 14·58; Cl, 24·65 per cent.)

Second Series

Allyl-thiocarbamido-8-aminoquinoline hydrochloride.

N·HCl

NH·CS·NH·CH₂·CH=CH₂
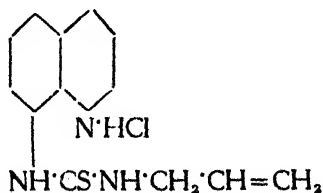
Aminoquinoline (1 g.) is mixed with allyl*iso*cyanate (1 g.) in methyl alcohol (5 c.c.). The mixture is warmed on water-bath for some time and then poured into water. The precipitate is dissolved in hydrochloric· acid, shaken ·with ether and the aqueous solution is made alkaline. The precipitate obtained is taken up with chloroform and the hydrochloride precipitated therefrom by passing HCl gas. It crystallises from alcohol and melts at 150°. It forms a yellow crystalline chromate with $K_2Cr_2O_7$ which does not melt below 300°. (Found : N, 15·20; Cl, 12·61. $C_{13}H_{14}N_3SCl$ requires N, 15·02 ; Cl, 12·70 per cent.)

Allyl-8-*aminoquinoline hydrochloride.*
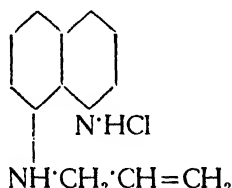
8-Aminoquinoline (1 g.) and allylbromide (1 g.) are mixed with Na_2CO_3 (2 g.) dissolved in 10 c.c. of water. The whole is refluxed for three hours. The mixture is shaken with ether and the hydrochloride is precipitated from the ethereal solution by passing HCl gas. It crystallises from alcohol, m.p. 175°. It gives with potassium dichromate solution a chromate which does not melt below 300°. (Found : N, 12·8; Cl, 11·50. $C_{12}H_{13}N_2Cl$ requires N, 12·7 ; Cl, 11·56 per cent.)

Allyl-thiocarbamido-8-amino-6-ethoxyquinoline   hydrochloride.
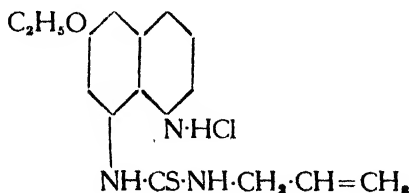
The starting materials in the preparation of this compound are 6-ethoxy-8-aminoquinoline and allyl*iso*thiocyanate. The former is obtained by reducing 6-ethoxy-8-nitroquinoline with $SnCl_2$ and conc. HCl, the nitroquinoline derivative itself being obtained by applying Skraup's reaction to 4-ethoxy-2-nitraniline (*loc. cit.*).

6-Ethoxy-8-aminoquinoline (2 g.) and an equivalent amount of allyl*iso*thiocyanate in 10 c.c. of methyl alcohol are warmed together on a water-bath for about an hour. The mixture is poured into water and purified as in the case of allylthiocarbamido-8-aminoquinoline. The hydrochloride is produced by passing dry HCl gas through its ethereal solution. It melts at 160° and forms a yellow dichromate which does not melt below 300°. (Found: N, 13·10; Cl, 11·10; $C_{15}H_{18}ON_3SCl$ requires N, 12·98; Cl, 10·97 per cent.)

Allyl-8-amino-6-ethoxyquinoline hydrochloride.
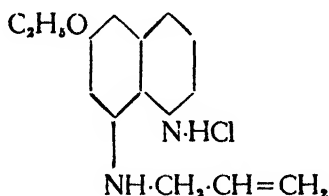
It is prepared by refluxing a mixture of 6-ethoxy-8-amino-quinoline (1 g.), allylbromide (1·1g.) and Na_2CO_3 (2 g.) dissolved in 8 to 10 c.c. of water. The refluxing is continued for 2 to 3 hours. It is then cooled and treated with ether. The hydrochloride is prepared as in the case of allyl-8-aminoquinoline; m.p. 182°. It forms a crystalline dichromate, which does not melt below 300°. (Found: N, 10·58; Cl, 13·32. $C_{14}H_{17}ON_2Cl$ requires N, 10·59; Cl, 13·42 per cent.)

N-(8-*quinolyl*)-μ-*Amino-α-methylthiazoline hydrochloride*.
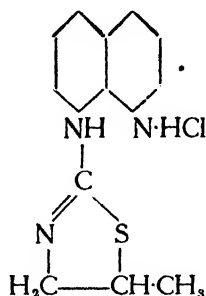
It is prepared by warming allylthiocarbamido-8-amino-quinoline with excess of concentrated hydrobromic acid on a water-bath for about an hour. The mixture is cooled and diluted with water and then made alkaline with caustic soda solution. The precipitate is next extracted with ether. The ethereal solution is dried over anhydrous K_2CO_3, and the hydrochloride prepared as usual. It melts at 215-20° (decomp.). It is a light yellow substance and dissolves with difficulty in water to a light yellow solution. (Found : N, 15·00 ; Cl, 12·62. $C_{13}H_{14}N_3SCl$ requires N, 15·02 ; Cl 12·70 per cent.)

STUDIES IN QUINOLINE COMPOUNDS
Part VII

In Part VI of the series of papers (*J. Indian Chem. Soc.*, 1932, 9, 37) we described the preparation of 6-methoxy-8-aminoisopropylaminoquinoline dihydrochloride which is structurally related to plasmoquine. It occurred to us that by ethylation we could enhance the similarity of this compound with plasmoquine and expected that they might possess some antimalarial properties. Other simpler aminoquinoline derivatives have also been synthesised with the same end in view.

Attempts to prepare the alkylated products by condensing β-bromopropylalkylamines with aminoquinolines were unsuccessful

EXPERIMENTAL

6-Methoxy - 8 - β - diethyl-aminoisopropyl-aminoquinoline dihydrochloride.

CH_3O

N·HCl

NH — CH $\langle$ CH_3 / CH_2·N(C_2H_5)$_2$·HCl
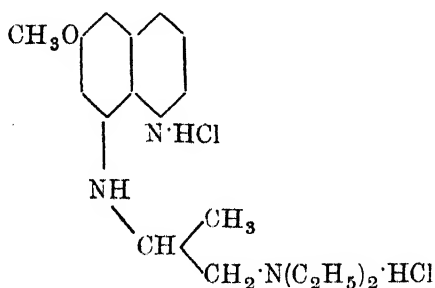
6-Methoxy-8-β-aminoisopropyl-aminoquinoline dihydrochloride (3·1 g.) (*loc. cit.*) was dissolved in water (15-20 c.c.)

and treated with anhydrous sodium carbonate (4 g.) when the corresponding base was precipitated as a viscous sticky mass. After the addition of a little more than the theoretical quantity of ethyl iodide the mixture was refluxed for $2\frac{1}{2}$ hours on a water-bath. The alkylated product floated as an oil and was extracted with ether. The ethereal solution was dried over anhydrous potassium carbonate and a dry current of hydrochloric acid gas passed through it when the dihydrochloride was precipitated but soon coalesced. The precipitate was next dissolved in a little absolute alcohol from which it crystallised out as a solid yellow crystalline substance readily dissolving in water to a clear yellow solution, m.p. 175°. (Found: N, 11·75; Cl, 19·53. $C_{17}H_{27}ON_3Cl_2$ requires N, 11·66, Cl, 19·72 per cent.)

6 - *Methoxy -8-β-dimethyl- aminoisopropyl-aminoquinoline dihydrochloride.* — 6-Methoxy-8-β-aminoisopropyl-aminoquinoline dihydrochloride (4·5 g.) dissolved in water (12 c.c.) was refluxed on a water-bath with N_2CO_3 (6 g.) and CH_3I (5·2g.) for 2 to 3 hours. The dihydrochloride was prepared as before. It is a yellowish brown crystalline compound easily dissolving in water, m.p. 180°. (Found : N, 12·70 ; Cl, 21·33. $C_{15}H_{23}ON_3Cl_2$ requires N, 12·65; Cl, 21·38 per cent.)

8-*β-Dimethyl-aminoisopropyl-aminoquinoline dihydrochloride* was prepared from 8-β-aminoisopropylaminoquinoline dihydrochloride (*loc. cit.*). It is a yellowish brown powder, m.p. 200-205°. (Found : N, 14·04 ; Cl, 23·40. $C_{14}H_{21}N_3Cl_2$ requires N, 13·90 ; Cl, 23·51 per cent.)

6 - *Methyl - 8- β - dimethyl-aminoisopropyl-aminoquinoline dihydrochloride* was also prepared from 6-methyl-8-aminoisopropyl-aminoquinoline hydrochloride, m.p. 210°. (Found : N, 13·22 ; Cl, 22·32. $C_{15}H_{23}N_3Cl_2$ requires N, 13·29; Cl, 22·46 per cent.)

2-*Methyl -6-methoxy-8-β-dimethyl-aminoisopropyl-aminoquinoline dihydrochloride* prepared from 2-methyl-6-methoxy-

8-amino*iso*propyl-aminoquinoline dihydrochloride in the usual way, melts at 218°. (Found: N, 12·00; Cl, 20·33. $C_{16}H_{25}ON_6Cl_2$ requires N, 12·14; Cl, 20·52 per cent.)

β-Hydroxypropyl- 8- *aminoquinoline   hydrochloride.*— Allyl-8-aminoquinoline hydrochloride (1 g.) (*loc. cit.*) was warmed with fuming HBr (5 c.c.) on a water-bath for 15 to 30 minutes. Excess of HBr was partly removed on the water-bath in a basin and the cooled mixture was next made alkaline. *β*-Bromopropyl-8-aminoquinoline was precipitated as an oil and was extracted with ether. The ethereal solution was dried and the ether removed when an oily substance was obtained which solidified on cooling and scratching and purified from alcohol.

A mixture of *β*-bromopropyl-8-aminoquinoline (1·2 g.) and water (101-2 c.c.) was gently boiled with Na_2CO_3 (2 g.) for 3 to 4 hours and the oil that separated was extracted with ether. The hydrochloride was prepared as usual and crystallised from alcohol. It melts at 170-72°.

That it is the *β*-hydroxy derivative follows from the fact it can as well be prepared by condensing chloro*iso*propyl alcohol with 8-aminoquinoline in the usual way. It is thus proved that it is the *β*-bromo-and *β*-hydroxy-compounds that are formed in the former method. (Found: N, 11·80; Cl, 15·00. $C_{12}H_{15}ON_2Cl$ requires N, 11·74, Cl, 14·88 per cent.)

6-Ethoxy-β- hydroxypropyl- 8-*aminoquinoline   hydrochloride.* —6 - Ethoxy-8 -*N* - allylaminoquinoline   hydrochloride (0·5 g.) was treated with fuming HBr (5 c.c.) and was then proceeded with as above. The hydrochloride melts at 165° and is hydrolysed by water. (Found: N, 10·02; Cl, 12·32. $C_{14}H_{19}O_2N_2Cl$ requires N, 9·91; Cl, 12·56 per cent.)

8-N-*Lactylaminoquinoline hydrochloride.*—8-Aminoquinoline (0·5 g.) and ethyl lactate (0·8 g.) were heated together in an oil-bath at 130° for $2\frac{1}{2}$ hours. The mixture was cooled, treated with dilute hydrochloric acid and shaken

with ether to remove oily insoluble matters. The aqueous solution was filtered, made alkaline and extracted with ether from which the hydrochloride was precipitated by passing dry HCl gas. It was further purified by crystallisation from alcohol. It is a light yellow crystalline powder which is hydrolysed by water, m.p. 182-85°. It also results from heating the lactate of 8-aminoquinoline at 175° for 2 hours. (Found : N, 11·21 ; Cl, 14·22. $C_{12}H_{13}O_2N_2Cl$ requires N, 11·09; Cl, 14·06 per cent.)

8-N-*Lactylamino-6-ethoxyquinoline hydrochloride* was obtained similarly from ethyl lactate and 6·ethoxy-8-amino-quinoline. The hydrochloride which hydrolyses with water melts at 177°. (Found : N, 14·02 ; Cl, 18·21. $C_{14}H_{17}O_3N_2Cl$ requires N, 14·25 ; Cl, 18·06 per cent.)

CHEMOTHERAPY OF QUINOLINE COMPOUNDS

Part I

A PRELIMINARY REPORT ON THE ACTION OF CERTAIN QUINOLINE COMPOUNDS ON PARAMŒCIA

The remarkable use of the alkaloids of cinchona bark, especially quinine, in the treatment of malarial fevers, makes the study of quinoline compounds a subject of unusual interest in chemotherapy. Until recently cinchona alkaloids were the only known compounds which had any effect upon malarial parasites. Quinine is a derivative of quinoline, being p-methoxy-λ-quinolyl-β-vinyl-2-quinuclidyl carbinol. It was originally supposed that quinine owed its therapeutic properties to the quinuclidyl nucleus. But Fraenkel afterwards put forward many facts in support of the view that it was the piperidine ring, the so-called " loiponic acid portion " of the molecule, which was the true " pharmacophore." The recent observations on the use of plasmoquine in the treatment of malaria, however, lead to the conclusion that the old idea was correct, and that it is the quinuclidyl nucleus which is responsible for its therapeutic properties. With this view the senior writer conceived the idea of studying certain quinoline derivatives and the present paper is a preliminary report of observations on the first series of such compounds which have been studied.

TABLE 1

Action of certain Quinoline Compounds on Paramœcia

	Strength	Effect on Paramœcia
I. 6-Amino-quinoline NH$_2$ (structure)	1 : 2,000 1 : 4,000	Death No death
II. Quinoline-6-glycine-amide NH$_2$·CO·CH$_2$·NH (structure)	1 : 2,000 1 : 4,000	Death No death
III. 8-Amino-quinoline NH$_2$ N (structure)	1 : 2,000 1 : 4,000	No death No death
IV. Quinoline-8-glycine-amide NH$_2$ CO·CH$_2$·NH N (structure)	1 : 2,000	No death
V. 6-Oxy-8-amino-quinoline HO (structure) NH$_2$ N	1 : 2,000 1 : 10,000 1 : 20,000 1 : 40,000 1 : 80,000 1 : 160,000 1 : 320,000	Death in 6 minutes Death in 7 minutes Death in 7 minutes Death in 8 minutes Death in 14 minutes Death in 17 minutes No death in 1 hour
VI. 6-Oxy-quinoline-8-glycine-amide HO (structure) NH$_2$·CO·CH$_2$·NH N	1 : 200 1 : 10,000 1 : 20,000 1 : 40,000 1 : 80,000 1 : 160,000 1 : 320,000	Death in 7 minutes Death in 7 minutes Death in 10 minutes Death in 11 minutes Death in 15 minutes Death in 19 minutes No death

TABLE 1 (*concluded*)

	Strength	Effect on Paramœcia
VII. 6-Methoxy-quinoline-8-glycine-amide	1 : 1,000	No action
Quinine hydrochloride	1 : 10,000 1 : 20,000 1 : 40,000 1 : 80,000 1 : 160,000	Death in 5 minutes Death in 10 minutes Death in 19 minutes Death in 35 minutes No death in 1 hour

For the structure in row VII: CH_3O attached to the quinoline ring system, with $NH_2 \cdot CO \cdot CH_2 \cdot NH$ and N.

For purpose of comparison we append here the action of quinine hydrochloride on paramœcia under conditions similar to the above.

In all the experiments given in Table 1 equal parts of the culture of paramœcia and of the solution of the compound were taken. The strength given in the table is that of the resulting mixture which is half of that of the original strength used. The proof of death of the paramœcia was indicated by their becoming at first motionless and subsequently disintegrated. The paramœcia were cultured on hay infusion.

Toxicity of 6-oxy-quinoline-8-glycine-amide.—Experiments show that orally 220 mgm. of the compound given to guinea pigs per kilogram of body weight on two successive days do not give rise to any toxic symptoms.

Conclusions

1. 6-Amino-quinoline and 8-amino-quinoline have no action on paramœcia in strength of 1 : 4000.

2. The introduction of OH into 8-amino-quinoline and quinoline-8-glycine-amide raises their toxic action on paramœcia to a remarkable degree.

3. The methylation of 6-oxy-8-aminoquinoline by replacement of H of OH by CH_3 reduces its action on paramœcia to nil.

4. Experiments show that orally 220 mgm. of 6-oxy-quinoline-8-glycine-amide given to guinea pigs per kilogram of body weight on two successive days do not give rise to any toxic symptoms.

5. Our results with 6-oxy-quinoline-8-glycine-amide and 6-methoxy-quinoline-8-glycine-amide are fairly comparable to those obtained in the case of oxy-quinoline, the properties of which decrease almost to zero when the hydroxy-group is methylated and these are exactly opposite to the data when quinine is compared physiologically with its demethylated compounds (Dyson).

Reference

Dyson, G. Malcolm :   The Chemistry of Chemotherapy, 1928.

CHEMOTHERAPY OF QUINOLINE COMPOUNDS

Part II

The Action of certain Quinoline Compounds on Paramœcia

The studies of certain quinoline compounds from a chemical point of view and their chemotherapy have been the subject of research of the senior writer and co-workers for some time.[1] These researches on quinoline compounds, as stated in this paper, have been conducted to discover such quinoline derivatives as would possess destructive action on paramœcia and subsequently to test their antimalarial properties, if any. At this time we were not aware that Robinson and co-workers were also conducting researches on the chemistry of certain quinoline compounds to find new antimalarials, their first paper on the subject being published in December, 1929.[2]

Though our researches and those of Robinson and co-workers have been conducted for similar purpose, yet the lines of both these investigations have been somewhat different from each other. We hope that both this work and that of Robinson and co-workers will be supplementary to each other, the common object being to discover some new quinoline antimalarials.

[1] The first paper of the authors on " Chemotherapy of Quinoline Compounds," which was communicated on September 19, 1929, to the Honorary Secretary, Royal Society of Tropical Medicine and Hygiene, is now under publication in the Journal of Pharmacology and Experimental Therapeutics.

[2] Journal of the Chemical Society, 1929, pp. 2947-51.

The first chemical paper on the researches of the senior writer (U. N. B.) and his collaborator (T. P. B.) in this direction was communicated to the Journal of the Indian Chemical Society on March 17, 1930 and is under publication.

The present paper on the chemotherapy of quinoline compounds gives an account of the action of a second series of compounds on paramœcia. In this paper two kinds of quinoline compounds have been studied.

1. Compounds allied to the derivatives of anil-quinoline which were found to possess strong antiseptic properties by Browning, Cohen, Ellingworth, and Gulbransen (1926). Of the series of these compounds a sulphonated derivative of No. 48 called " 48 S " has been recently prepared and suggested to be named quinanil (Armitage, Gordon, Cohen, Ellingworth and Dobson, 1929). It has been also recently found by these observers to have a high antiseptic titer with a low toxicity and to be valuable in the treatment of surgical infections.

The compounds of this series tested by us are :

(*i*)   2-*p*-Dimethyl-amino-styryl-quinoline hydrochloride.

(*ii*)   6-Oxy-2-*p*-dimethyl-amino-styryl-quinoline.

(*iii*)   6-Ethoxy-2-*p*-dimethyl-amino-styryl-quinoline.

(*iv*)   6-Methyl-2-*p*-dimethyl-amino-styryl-quinoline-methiodide.

(*v*)   2-*p*-Dimethyl-amino-anil-quinoline.

(*vi*)   *p*-Dimethyl-amino-benzal-6-amino-quinoline.

None of the above compounds has any action against paramœcia in dilution of 1 : 2,000 and upwards in water.

2. Compounds derived from 4-phenyl-quinaldine. The enhanced toxicity of the latter towards certain kinds of protozoa, when compared with that of quinoline and quinaldine, induced us to study some of its derivatives.

Two new compounds of this series synthesized by some of us (U. N. B. and T. P. B) have up to now been tested by us to determine their action on paramœcia. They are 6-amino-4-phenyl-quinaldine hydrochloride and 8-amino-4-phenyl-quinaldine hydrochloride. Table 1 gives the result of their toxicity towards paramœcia.

Two of the compounds, *i.e.*, 6-oxy-8-amino-quinoline and 6-oxy-quinoline-8-glycine-amide tested by us in the treatment of malarial fevers, while possessing very toxic action against paramœcia, possess no therapeutic properties in the treatment of benign tertian or malignant tertian malaria. The two new derivatives of phenyl-quinaldine described in this paper have not yet been tested in the treatment of malaria.

TABLE 1

Action of certain Quinoline Compounds on Paramœcia

	Strength	Effect on paramœcia
I. 6-Amino-4-phenyl-quinaldine hydrocholoride	1 : 2,000 1 : 10,000	No death in 1 hour No death in 1 hour
II. 8-Amino-4-phenyl-quinaldine hydrochloride	1 : 2,000 1 : 10,000 1 : 20,000 1 : 40,000	Death in 4 minutes Death in 24 minutes Few died in 1 hour No death in 1 hour

The following new quinoline compounds have been found to possess marked action against paramœcia :

(*i*) 6-Oxy-8-amino-quinoline. (*Vide* Chemotherapy of Quinoline Compounds, Part I.)

(*ii*) 6-Oxy-quinoline-8-glycine-amide. (*Ibid.*)

(*iii*) 8-Amino-4-phenyl-quinaldine hydrochloride.

The compounds allied to those described by Browning and co-workers and which have so far been investigated by us do not possess any action against paramœcia.

Further investigations are in progress.

CHEMOTHERAPY OF QUINOLINE COMPOUNDS

PART III

The Action of certain Quinoline Compounds on Paramœcia

This communication is a continuation of previous papers on the action of certain quinoline compounds on paramœcia investigated by some of us and collaborators and which were published in this Journal (Vol. XXXIX, No. 4, August, 1930, and Vol. XLI, No. 3, March, 1931). It gives record of our observations on a further series of new quinoline compounds, the chemistry of which, including that of others, has been studied by some of us in papers which have been published in the *Journal of the Indian Chemical Society* (Vol. VII, Nos. 6, 9 and 10; Vol. VIII, Nos. 1 and 6); and others are under publication in the same journal. For reference the list of these compounds is given below :

(1) 2-*p*-Dimethyl-amino-styryl-quinoline.

(2) 6-Methyl-2-*p*-dimethyl-amino-styryl-quinoline.

(3) 6-Oxy-2-*p*-dimethyl-amino-styryl-quinoline.

(4) 6-Ethoxy-2-*p*-dimethyl-amino-styryl-quinoline.

(5) 6-Methoxy-2-*p*-dimethyl-amino-styryl-quinoline.

(6) Quinoline-6-amino-acetamide.

(7) Quinoline-8-amino-acetamide.

(8) 6-Ethoxy-quinoline-8-amino-acetamide.

(9) 6-Methoxy-quinoline-8-amino-acetamide.

(10) 6-Nitro-4-phenyl-2-methyl-quinoline.

(11) 6-Amino-4-phenyl-2-methyl-quinoline.

(12) 6-Nitro-4-phenyl-2-*p*-dimethyl-amino-styryl-quinoline.

(13) 8-Nitro-4-phenyl-2-methyl-quinoline.

(14) 8-Amino-4-phenyl-2-methyl-quinoline.

(15) 8-Nitro-4-phenyl-2-*p*-dimethyl-amino-styryl-quinoline.

(16) Quinoline-8-amino-acetyl-*p*-arsanilic acid.

(17) 6-Methoxy-quinoline-5-amino-acetyl-*p*-arsanilic acid.

(18) Quinoline-6-amino-acetyl-*p*-arsanilic acid.

(19) 2-Methyl-quinoline-6-amino-acetyl-*p*-arsanilic acid.

(20) 8-Amino-quinoline-*p*-arsanilate.

(21) 6-Amino-quinoline-*p*-arsanilate.

(22) Sodium-8-amino-quinoline-*N*-methylene-sulphonate.

(23) Sodium-6-amino-quinoline-*N*-methylene-sulphonate.

(24) Sodium-6-methoxy-8-amino-quinoline-*N*-methylene-sulphonate.

(25) Sodium-8-amino-quinoline-*N*-methylene-sulphinate.

(26) Sodium-6-amino-quinoline-*N*-methylene-sulphinate.

(27) Sodium-6-methoxy-8-amino-quinoline-*N*-methylene-sulphinate.

(28) 8-Carbamido-quinoline.

(29) 6-Carbamido-quinoline.

(30) 6-Methoxy-8-carbamido-quinoline.

(31) 8-Amino-*iso*propyl-amino-quinoline.

(32) 6-Methyl-8-amino-*iso*propyl-amino-quinoline.

(33) 6-Methoxy-8-amino-*iso*propyl-amino - quinoline di-hydrochloride.

(34) 6-Ethoxy - 8- amino-*iso*propyl - amino-quinoline di-hydrochloride.

(35) 8-Amino-ethyl-amino-quinoline.

(36) 8-Amino-propyl-amino-quinoline.

(37) 2-Methyl-6-methoxy - 8 - amino - *iso*propyl - amino-quinoline dihydrochloride.

(38) 2-Methyl-6-ethoxy-8-amino-*iso*propyl - amino- quinoline dihydrochloride.

(39) 2- Methyl-6-chloro-8-amino - *iso*propyl-amino - quino-
line dihydrochloride.

(40) 6-Chloro-8-amino - *iso*propyl - amino - quinoline di-
hydrochloride.   •

(41) 2 - Methyl -8- amino - *iso*propyl - amino - quinoline di-
hydrochloride.   •

(42) Allyl - thiocarbamino- 8- amino - quinoline   hydro-
chloride.

(43) Allyl-8-amino-quinoline hydrochloride.

Incidentally we have noted that piperidine-para-arsanilate
possesses no action on paramœcia in strength of 1 : 2,000
and 1 : 10,000.

TABLE I

Action of certain Quinoline Compounds on Paramœcia

	Strength	Effect on paramœcia
1. 8-Amino-quinoline-para-arsanilate	1 : 2,000 1 : 10,000	Few died in 1 hour No death in 1 hour
2. 6-Amino-quinoline-para-arsanilate	1 : 2,000 1 : 10,000	No death in 1 hour No death in 1 hour
3. 8-Amino-ethyl-amino-quinoline hydrochloride (Robinson)	1 : 2,000 1 : 10,000 1 : 20,000	Death in 3 minutes Death in 29 minutes No death in 1 hour
4. 8-Amino-*iso*propyl-amino-quinoline hydrochloride	1 : 2,000 1 : 10,000 1 : 20,000 1 : 40,000	Death in 20 minutes Death in 35 minutes 90 per cent died in 1 hour No death in 1 hour
5. 6-Methoxy-8-amino-*iso*propyl- amino-quinoline dihydrochloride	1 : 2,000 1 : 10,000	Few died in 1 hour No death in 1 hour
6. 6-Chloro-8-amino-*iso*propyl-amino- quinoline dihydrochloride	1 : 2,000 1 : 10,000 1 : 20,000 1 : 40,000 1 : 80,000 1: 100,000	Death in 1 minute Death in 4 minutes Death in 12 minutes Death in 30 minutes No death in 1 hour No death in 1 hour
7. 6-Chloro-2-methyl-8-amino-*iso*propyl- amino-quinoline dihydrochloride	1 : 2,000 1 : 10,000	Death in 30 minutes No death in 1 hour
8. Amino-acetyl derivative of 8-amino- ethyl-amino quinoline	1 : 2,000 1 : 10,000	90 per cent died in 1 hour No death in 1 hour

The chemistry of quinoline compounds studied by Brahmachari and co-worker has been published in the *Journal of the Indian Chemical Society*.
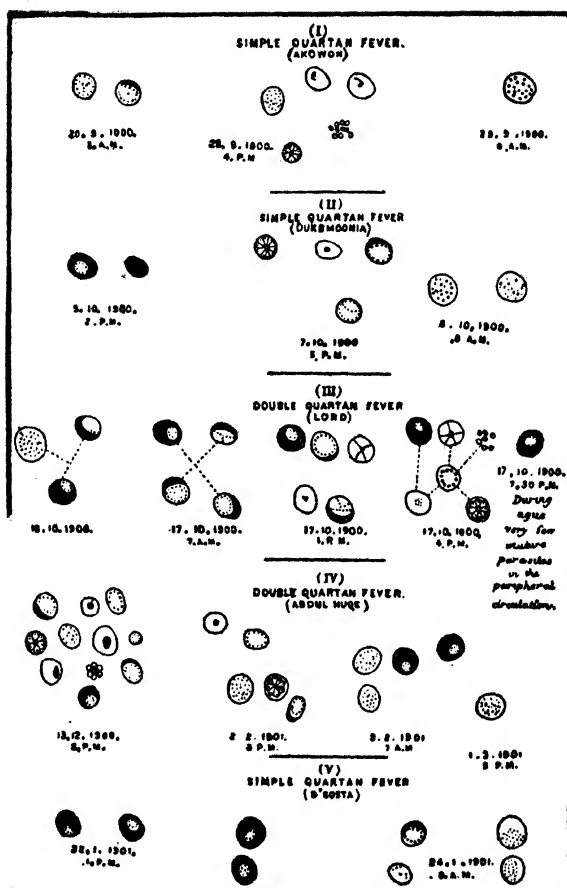
Parasites of quartan fever in different stages of the fever drawn by camera lueida (reduced in size).

FIVE CASES OF QUARTAN FEVER

The following cases of quartan fever were treated in the wards of the 1st Physician at the Medical College Hospital, Calcutta, between September, 1900 and February, 1901. The diagrams of the parasites observed in each case are appended.

Case No. 1.—Akawon, Chinaman, æt. 30, was admitted on 23rd September, 1900. Patient was employed in Jalpaiguri in the Rajshahi Division in a tea garden during 1899. While there, he was attacked with malarial fever for the first time and was treated with quinine. He came to Calcutta in May, 1900 and suffered from fever time to time, and as the attacks became more frequent he came into hospital.

Case No. 2.—Dhookmooia, Hindu, female, æt. 16, was admitted on 2nd October, 1900, suffering from malarial fever. Patient was employed in 1899 in Lilooah, a station three miles from Howrah, as a labourer in the railway construction works for about eight months. While there, she was attacked with intermittent fever. She came to Calcutta in June, 1900, and was suffering from malarial fever since then.

Case No. 3.—Lord, European, male, adult, was admitted on 13th October, 1900. He had the first attack of malarial fever in Hyderabad (Deccan) in 1899, and was admitted for treatment into the Residency Hospital in June, 1899, and remained there for about a fortnight. He was re-admitted there for thes ame complaint in January, 1900, and remained in hospital for about three weeks. He came to Calcutta from Hyderabad in February, 1900. While in

Calcutta he had frequent attacks of fever and eventually sought admission into hospital.

Case No. 4—Abdul Huq, Mahomedan, male, æt. 16, was admitted on 27th November, 1903, for the treatment of hemiplegia. He was in Midnapur for about eight days in 1899 and then in Calcutta, where he had the first attack of intermittent fever after staying three days. He was treated with quinine. He then went to Burdwan and stayed there for about a fortnight. He then lived in a village called Golgram, about six miles from Burdwan, where he frequently suffered from malarial fever. He came to Calcutta for the treatment of hemiplegia in November, 1900.

Case No. 5.—D'Costa, Eurasian, male, æt. 43, was admitted on 19th January, 1901, complaining of fever and tremors in the right hand and in both the legs. Patient was in Hughli in 1894 for about a month, and had been suffering from intermittent fever since 1895, the attacks coming on between September and February. He had since then often been in Hughli, his last visit to the place being about two months previous to the present attack, which began on the 14th of January.

As the patient presented some very abnormal nervous symptoms, the notes of the case are given at full length.

On admission, the patient was found suffering from tremors of both the legs and of the right hand in which they were most marked. The tremors began after a fit of ague on the 14th. They were somewhat continuous ; diminishing during rest and increasing during action. There was nothing characteristic about the speech. The knee jerks were markedly increased and ankle clonus was distinctly present on both sides. There were exophthalmos in both the eyes and slight paresis of the right hand, the dynamometer showing 20 in the right and 30 in the left hand. On 21st January, 1901, the tremors disappeared but the increased knee jerks with exophthalmos and ankle clonus persisted.

On the 23rd January, the exophthalmos had markedly diminished, and the tremors and ankle clonus had all disappeared. The increased knee jerks were still present. On the 25th, the patient left hospital against advice. At the time of discharge the exophthalmos was very slightly perceptible and the other nervous symptoms had all disappeared.

REMARKS

Quartan fever is considered to be very rare in India. In his presidential address on the fevers of India in the Indian Medical Congress, Dr. Crombie remarked that he had seen only one case in his whole experience in India. Dr. Maynard recorded one doubtful case in an out-patient in the Medical College Hospital, Calcutta. Ross found only two patients suffering from quartan fever out of 112 cases of malarial fever examined by him in the Madras Infantry. The following table, mostly taken from Mannaberg's *Treatise on Malarial Fevers* shows the number of observations made in the other parts of the world by different observers :—

Name of observer		Number of Quartans		Total number of cases of malarial fever observed		Seat of observation
Moillot	...	26	...	2,338	...	Algiers
Koch	...	15	...	408	...	Italy
Finah	...	21	...	4,211	...	Algiers
Durand	...	6	...	625	...	Tunis
Osler	...	5	..	616	...	Baltimore
Laveran	...	7	...	311	...	Algiers
Griesenger	...	3	...	414	...	Tubenger
Thayer and Hewetson	...	5	...	542	...	Johns Hopkins Hospital

Double quartan fever is considered by many to be quite uncommon. Mannaberg quotes from authors who deny its existence. He observed only two cases in his own experience. Out of 77 cases of malarial fever, the notes of the blood examination of which have been kept by me and which were

observed in the wards of the 1st Physician, Medical College Hospital, Calcutta, there were five cases of quartan fever, two of which were double quartans.

In all the five cases the quartan parasites were recognised in the blood, characterized by (a) their sharp contour and refractile nature, (b) the presence of coarse pigment granules which were only slightly motile and more or less peripherally distributed except in the swollen extra-corpuscular forms in which the pigment was irregularly distributed and in dancing motion, (c) the small size of the full-grown parasites, and especially of the extra-corpuscular bodies as compared with the tertian ones, and (d) the absence of expansion of any of the infected red blood corpuscles which were even sometimes smaller than normal and never lost their contour. The characteristic rosettes were seen in all except in Case No. 5, in whose blood, however, they were not looked for at the time when they generally begin to develop. The spores within the rosettes never exceeded 10 in number, though they were sometimes as few as 6 (vide diagrams). The arrangement of the spores in the segmenting forms was quite characteristic and differed from what is found in those of the tertian parasites.

The segmenting forms well merit the term " Marguerite " or rosette forms so frequently applied to them. Occasionally, the rosettes showed some peculiar evolution while on the slide under the microscope, but their changes have not been shown in the diagrams. While observing the parasites in one case under the microscope I noticed that the pigment granules after remaining clumped up in the centre of a rosette for twenty-four hours, commenced a most active movement (well described as a " boiling movement ") which continued for ten days. The spores within the rosette became very indistinct after forty-eight hours, so that it was practically converted into a more or less hyaline spherical body with a clump of very actively moving pigment granules

in the centre. After ten days the parasite disintegrated, leaving the pigment free. The slide containing the specimen of the blood was kept in the wards, the range of temperature being from 80°F. to 85°F. The cover glass was ringed with vaseline to prevent the blood drying up.

On one occasion a rosette was seen, to rupture within an hour after the blood had been drawn, and in several other cases a similar phenomenon was observed at a later period, generally within twelve hours. The spores let free did not appear to attack any of the red corpuscles. In the process of rupture, the rosette got bigger and the spores seemed to have an active motion.

The appearance of the parasites after a course of cinchona febrifuge or quinine has not been shown in the diagrams. After a course of these drugs the parasites often looked fatty, and the peripheral distribution of the pigment granules in them was not so well marked.

In Case No. 4, rosettes were found on 19th January, 1901, though there was no fever on that date. This apyrexia was not due to treatment with quinine or cinchona febrifuge and remains unexplained. It shows that sometimes in the course of intermittent fever periods of apyrexia may occur spontaneously. In this case also, the parasites that were found in the blood on 1st March, 1901, were present for a long time after the patient had quinine and after the fever had ceased. These parasites may be considered to undergo further evolution in the mosquito. They were still present in the blood of the patient on the day of his discharge from hospital after he had been treated with quinine for more than a fortnight.

Some of the above cases presented some further points of clinical importance which may be mentioned here.

Case No. 2, after a course of cinchona febrifuge, had a period of apyrexia for some time and then began to suffer from a type of fever at first intermittent and then remittent.

During these attacks the blood was examined on several occasions but no malarial parasites were found. This latter fever did not yield to quinine or cinchona febrifuge and ended fatally.

The *post-mortem* examination showed no tubercle in the lungs or any other organ of the body. There was no evidence of typhoid fever. The mesenteric glands were enlarged, and a section showed a large number of pigmented leucocytes. The liver was very fatty, and the spleen showed an increase of fibrous tissue with a very large number of pigmented leucocytes. The *post-mortem* diagnosis of the case was " chronic malaria." The case shows how some of the intermittent fevers may become remittent, resist quinine and end fatally. It is possible that many of the remittent fevers of India begin with such intermittent attacks, and such a termination of intermittent fever is difficult to explain by the parasite theory.*

Case No. 4 is also interesting. The temperature chart exhibited a double quartan fever with a simple quartan relapse. It supports the statement of many observers that quartan fever is the most obstinate of all the intermittent fevers. The case shows the beneficial effect of small doses of quinine in the treatment of quartan fever as has been suggested by Legrain.[1]

Case No. 5 is unique in the nervous symptoms manifested by him. Cases simulating disseminated sclerosis have been recorded as being due to the æstivo-autumnal parasite,[2] and exophthalmos has been recorded to have been caused by the tertian parasite.[3] No such symptoms have, as far as

* The subsequent history of Case No. 2 in the present paper appears to point out that it ended in an attack of kala-azar—a disease which was in those days frequently mistaken for malaria. The resistance to quinine or cinchona and the fatal termination are thereby easily accounted for.

[1] Legrain's Fievres Des Paya Chands.

[2] American Journal of Medical Science, December, 1900.

[3] Knies' Eye in General Diseases.

I am aware, been shown to have resulted from a quartan
parasite infection.   The temperature chart was also interest-
ing as, instead of steadily coming down the next day after
ague, the temperature showed only, a morning fall and then
a rise resembling the curve of malignant tertian fever as
described by Marchiafava and Bignami.[4]   The fits of ague
came on, however, every fourth day.   The blood showed
an unusually large number of quartan parasites, and the
nervous symptoms may possibly be thus accounted for.   The
case may be described as one of quartan fever with somewhat
malignant symptoms.

   A point of great interest in the case is that the patient
suffered from intermittent fever every year between the
months of September and February—the time in fact during
which all the other cases were admitted into the hospital.
These months are apparently those during which this type
of intermittent fever is most prevalent.

   Since the above was written, another case was admitted
into the hospital under my care, the patient being a
Chinaman and coming from Jalpaiguri.

[4]   Thayer's Malarial Fevers.

References

Transactions of the Indian Medical Congress, 1895.
Indian Medical Gazette, November, 1895.
Ibid., 1896.
Legrain's Fievres Des Paya Chands.
American Journal of Medical Sciences, December, 1900.
Knies' Eye in General Diseases.
Thayer's Malarial Fevers.

   The drawings were made with the assistance of Abbe's Camera Lucida from
specimens of fresh blood with an Objective 1 12 (Oil immersion) and Ocular
No. 2.

   Note.—Quartan fever, though the least common of all malarial fevers, is not so
uncommon as was considered by Crombie.   In the Indian Medical Research Memoirs
(No. 18, December, 1930), Knowles and Senior White point out that out of 697 cases
of malarial fever with positive finding of parasites in the blood, 10 per cent. showed
infection with the parasites of quartan fever (P. malariæ).

QUARTAN FEVER IN CALCUTTA AND DACCA

The present paper is a continuation of the series of five cases of quartan fever which were published in the August number (1900) of the *Indian Medical Gazette*.

Case No. 6.—Alam, Chinaman, æt. 28, was admitted into the wards of the First Physician, Medical College Hospital, Calcutta, on 23rd April, 1901. Patient came from Jalpaiguri where he was attacked with malarial fever for the first time and was treated with quinine. The temperature chart exhibited a simple quartan fever tending to become double.

Case No. 7.—Baldao, Hindu, æt. 50, was admitted into the wards of the First Physician, Medical College Hospital, Calcutta, on 4th May, 1901. Patient was a pilgrim going to the temple of Jagannath. He was for some time in Burdwan where he was attacked with intermittent fever. The temperature chart exhibited a triple quartan fever being converted into the simple quartan type due to small doses of quinine. There was also a well-marked retardation of the paroxysms due to quinine.

Case No. 8.—Idatulla, Mahomedan, æt. 30, was admitted into my wards in the Mitford Hospital, Dacca, on 24th July, 1901. Patient came from Mymensingh where he had the first attack of malarial fever about three years ago and suffered from time to time since. The temperature chart exhibited a double quartan fever being spontaneously converted into the simple type due to spontaneous destruction or weakening of a mild set of quartan parasites.
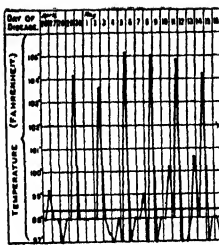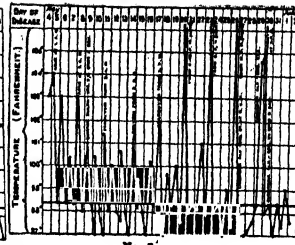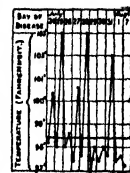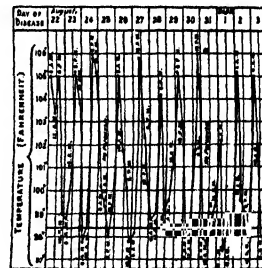
Quartan fever in Calcutta and Dacca

Case No. 9.—Badri, Hindu, æt. 25, was admitted into my wards in the Mitford Hospital, Dacca, on 5th September, 1901, in a state of extreme anæmia. The temperature chart showed a triple quartan fever due, to the presence of one set of strong and two sets of mild quartan parasites. The extreme anæmia led to the suspicion of ankylostomiasis, but the examination of the stools gave negative results.

Case No. 10.—Bachu, Mahomedan, æt. 20, was admitted into my wards in the Mitford Hospital, Dacca, on 21st August, 1901. Patient came with history of having suffered from intermittent fever for about a month. The temperature chart was that of an irregular type of intermittent fever with no paroxysms for two days and quotidian attacks for several days. There were marked retardations and anticipations of the paroxysms like what we have in æstivo-autumnal fevers. The blood contained a very large number of quartan parasites in various stages of development. The temperature chart is a 6-hour one.

Besides the above, there was another case of simple quartan fever in the wards of the First Physician, Medical College Hospital, Calcutta. The patient was nephew of patient No. 5 with whom he lived since his boyhood. He came with a history of intermittent fever for about three years.

Remarks.—All the cases recorded above were chronic ones with well marked enlargement of the spleen. Some of the cases, namely, Lord, Dhookmoonia and Badri, came with extreme anæmia.

In studying cases of quartan fever, it is common to find a tendency towards conversion of one type of the fever into another (*vide* charts *vi* and *viii*). This peculiarity is, I think, characteristic of quartan fever. The quartan parasite is characterized by being the mildest and at the same time the most obstinate of all the malarial parasites. Due to its mild nature a set of quartan parasites may be so much weakened

spontaneously as to be able to give rise to no clinical symptoms for some time. In this way a double quartan fever may be converted into the simple variety. Then, again, due to its obstinacy, the same set of parasites in the process of "their ordinary cycle of development may eventually reach a number sufficient to produce again the characteristic clinical symptoms." Thus a double quartan fever may be spontaneously converted into the simple variety, and then after a time be reconverted into the original type without any re-infection. This change of type is, I think, more common than has hitherto been observed.

The following is a list of all the cases that have come under my observation :—

1.	D'Costa	...	Simple quartan fever of a peculiar type.
2.	D'Costa (nephew to above)	...	Simple quartan.
3.	Akawon	...	Ditto.
4.	Lord	...	Double quartan.
5.	Abdul Huq	...	Ditto. (becoming simple due to quinine).
6.	Dhookmoonia	...	Simple (becoming remittent and terminating fatally).
7.	Alam	...	Simple (becoming double).
8.	Idatulla	...	Double (becoming simple).
9.	Baldao	...	Triple (becoming simple due to quinine).
10.	Badri	...	Triple.
11.	Bachu	...	Irregular (quotidian for some days).

SOME OBSERVATIONS ON BLOOD PRESSURE DURING INTRAVENOUS INJECTION OF QUININE IN THE TREATMENT OF MALARIAL FEVER

In the *Indian Journal of Medical Research* for January, 1919, McCarrison and Cornwall pointed out that all salts of quinine produce a profound fall of blood pressure in the sheep after intravenous injection. Many observers have advocated the treatment of malarial fever with intravenous injection of concentrated solutions as a perfectly safe method of administering the alkaloid. Recently, this method was frequently used in the war in Mesopotamia and other places where troops were attacked with malaria.

So far as I am aware, there are no systematic records of observations on blood pressure in man during treatment with intravenous injection of quinine during an attack of malarial fever. My observations were made in the wards of the Campbell Hospital, Calcutta.

It may be pointed out here that during attacks of malarial fever the blood pressure is generally low, especially in the pernicious type of cases. At the same time, it is in these latter cases that one looks for introducing quinine into the system in the shortest time, and therefore feels tempted to administer the drug by the intravenous method. If, at the same time, there is a profound fall of blood pressure during its administration, then the operation is dangerous and may

even prove fatal. Cases of death following intravenous administration of quinine are rarely reported or are attributed to causes other than the operation itself. A recent author has attributed cases of sudden death in such cases to introduction of large quantities of saline and considers that quinine should always be given in a concentrated solution. (Alport.)

It is to determine the changes in blood pressure during intravenous injection of quinine in an attack of malarial fever that the following observations were made. The injections were generally given in the pyrexial period except in one case. In all cases quinine bihydrochloride dissolved in normal solution was used.

I give here a summary of the observations made in each case :—

(1) Patient, Nirmala, suffering from recurring quartan fever.

Temperature—normal at the time of injection.

Curve (1) shows that there was not much fall of blood pressure when quinine was given in a dose of 10 grains dissolved in 200 c.c. of saline and at the rate of 10 c.c. per minute.

Curve (2) also shows that, as in the first case, there was very little change in the blood pressure when quinine was injected in a 10-grain dose dissolved in 200 c.c.

Curve (3) shows that there was a sudden fall of blood pressure when quinine was injected in a 10-grain dose dissolved in 200 c.c. in 3 minutes and 30 seconds.

Curve (4) shows that, immediately after injection of a concentrated solution in 15 seconds, the patient became pulseless for some seconds and there were muscular twitchings, the blood pressure rising to 70 after 2 minutes.

(2) Patient, Matidar, suffering from recurring attacks of benign tertian fever.

Temperature—normal at the time of injection.
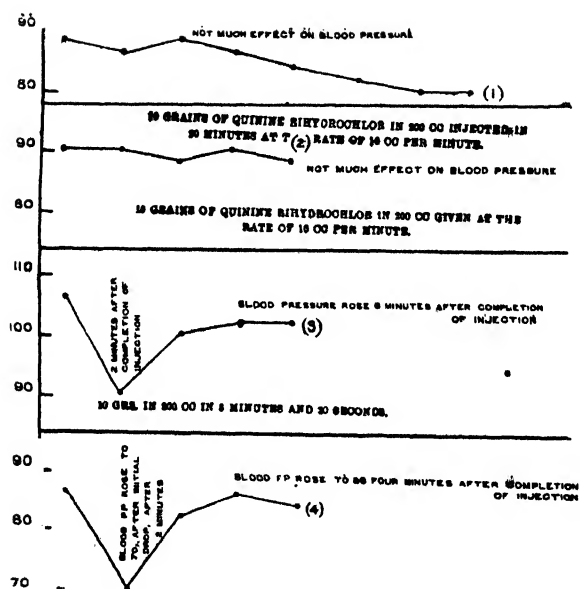
Blood pressure during intravenous injection of quinine

Case No. 1
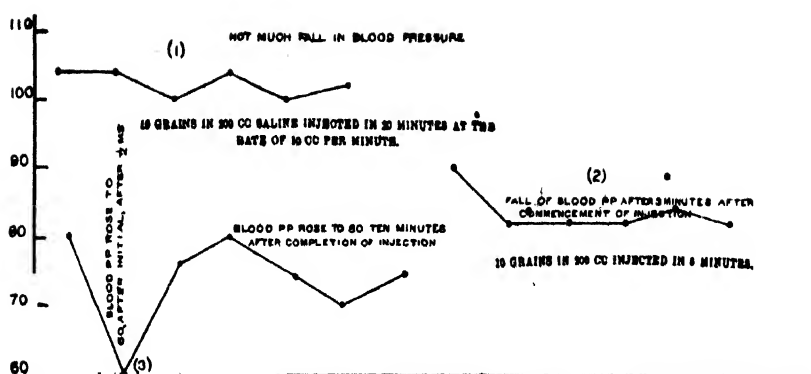
Blood pressure during intravenous injection of quinine

Case No. III
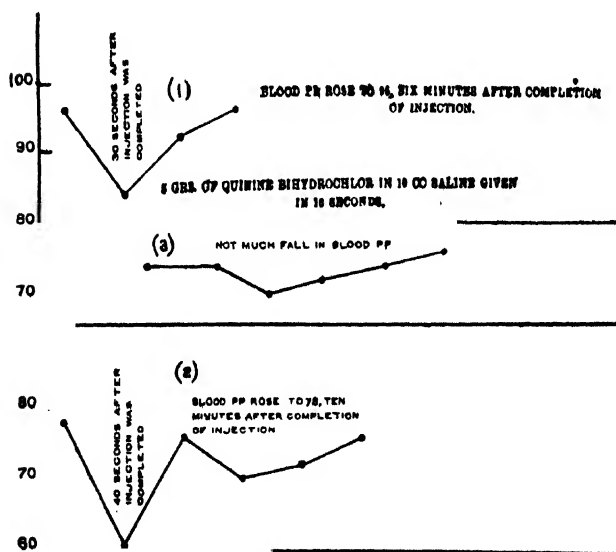
Blood pressure during intravenous injection of quinine

Case No. IV
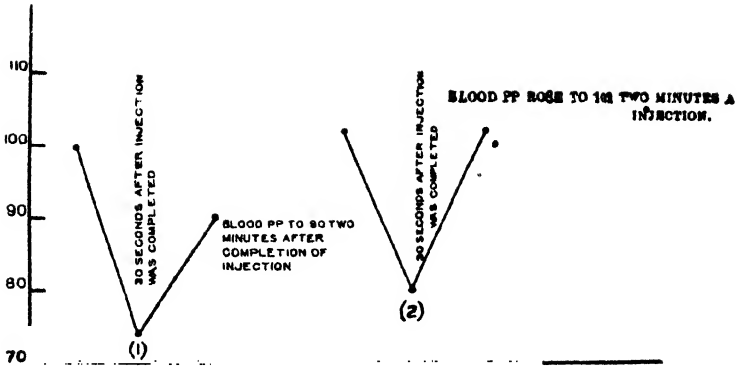
Blood pressure during intravenous injection of quinine
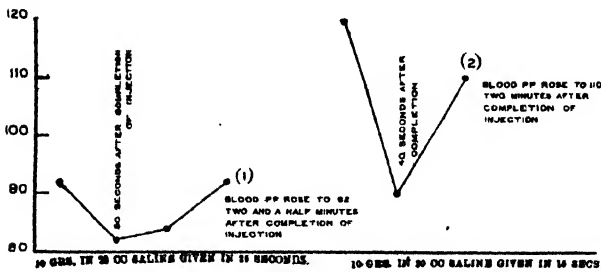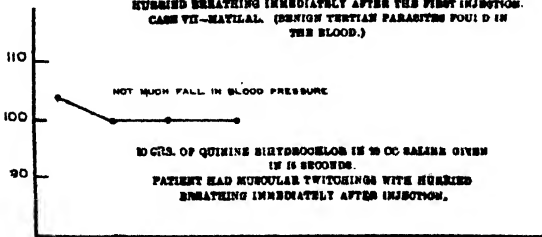
Case No. V

Blood pressure during intravenous injection of quinine
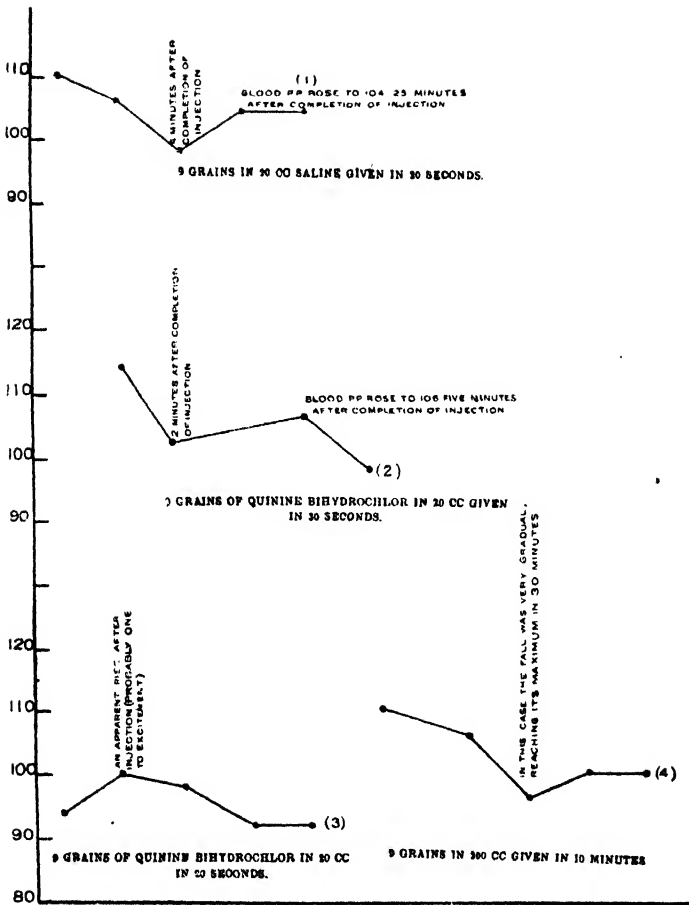
Case No. VI

Blood pressure after intravenous injection of quinine

Case No. II

Curve (1) shows that there was a drop of 12 mm. when quinine was injected in 20 seconds in a 9-grain dose dissolved in 20 c.c.

Curve (2) shows that there was a drop of 12 mm. when quinine was injected in 30 seconds in a 9-grain dose dissolved in 20 c.c.

Curve (3) shows that there was an apparent rise of blood pressure after the injection of quinine in a 9-grain dose dissolved in 20 c.c. This was perhaps due to excitement (at any rate, this rise of blood pressure after injection of quinine was not noticed in any other case).

Curve (4) shows that there was a gradual fall of blood pressure, reaching its maximum in 30 minutes, when quinine was injected in 10 minutes in a 9-grain dose dissolved in 200 c.c. The blood pressure remained for some hours 10 mm. lower than what it was before injection.

(3) Patient, Purna, suffering from recurring benign tertian fever.   •

Temperature—normal at the time of injection.

Curve (1) shows that there was not much fall of blood pressure when quinine was injected in 20 minutes in a 10-grain dose dissolved in 200 c.c.

Curve (2) shows that there was a fall of 8 mm. when quinine was injected in 8 minutes in a 10-grain dose dissolved in 200 c.c.

Curve (3) shows that the patient was pulseless for 10 seconds when quinine was injected in 25 seconds in a 6-grain dose dissolved in 20 c.c. The blood pressure rose to 60 mm. one-and-a-half minutes after the initial fall, slowly rising to 80 mm.

(4) Patient, Laha Singh, suffering from malignant tertian fever with crescents in the blood.

Curve (1) shows that there was a drop of 12 mm. when quinine was injected in 10 seconds in a 5-grain dose dissolved in 10 c.c. the pressure rising almost to the original

height six minutes after completion of the injection. (Injection given when temp. was 103° F.)

Curve (2) shows that there was a drop of nearly 20 mm. when quinine was injected in 15 seconds in a 10-grain dose dissolved in 20 c.c. (Injection given when temp. was 99·6°F.)

Curve (3) shows that there was very slight fall of blood pressure when quinine was injected in 10 minutes in a 10-grain dose dissolved in 200 c.c. (Injection was given when temp. was 96·4°F.)

(5) Patient, Yakub, suffering from recurring quartan fever.

The curves show that there was a drop of 22 to 26 mm. when quinine was injected in a 10-grain dose in 15 to 20 seconds after being dissolved in 20 c.c.

(6) Patient, Ascari, suffering from recurring benign tertian fever.

The curves show that there was a drop of nearly 10 to 30 mm. when quinine was injected in a 10-grain dose in 15 to 25 seconds after being dissolved in 20 c.c.

(7) Patient, Motilal, suffering from benign tertian infection.

Temperature—normal at the time of injection.

There was not much fall of blood pressure when quinine was injected in a 10-grain dose in 15 seconds after being dissolved in 20 c.c. The patient, however, had muscular twitchings and hurried breathing immediately after injection.

A paper similar to the above entitled *A Note on Blood Pressure during Intravenous Injection of Quinine* was published in the Lancet in December, 1920. (Editor.)

DANGERS OF RAPID INTRAVENOUS INJECTION OF QUININE SOLUTION

In a paper published in *The Lancet* as well as in the *Indian Medical Gazette* two years ago [1] I pointed out that there may be a dangerous fall of blood pressure after injection of concentrated solution of quinine in the treatment of malarial fever. Since then I have made further observations and have confirmed my former conclusions. On the other hand, some observers have pointed out that intravenous injection of large quantities of saline, such as a pint, may prove too much for a weak heart. I have also observed that there may be a dangerous fall of blood pressure during rapid intravenous injection of dilute or concentrated solutions of quinine. The whole problem depends upon the amount of quinine passing through the heart every second; and therefore, the slower the injection, there is less chance of a profound fall of blood pressure. Besides, if the rate of injection of the diluting fluid be the same, a greater quantity of quinine will be passing through the heart each second if the solution is concentrated than when it is dilute.

The problem divides itself into the following parts : (1) How much saline may be safely injected into a severe case of malaria, where the patient's blood pressure is low, say, 60 or 70mm., or when he is absolutely collapsed ? (2) Is there any advantage or disadvantage in diluting the quinine

[1] The Lancet, ii, and the Indian Medical Gazette, lv, 1920.

solution beyond a certain limit ? (3) Is there any danger in using the drug in a concentrated solution, using, say, 15 gr. in 5 c.c. and completing the injection in 75 to 100 seconds? In my opinion the amount of quinine injected into a vein at the bend of the elbow should not be more than 1/120 gr. per second or $\frac{1}{2}$ gr. per minute. This will mean that 10 gr. will take 20 minutes for completion of the injection. If one uses a dilution of 1 in 300, this will mean that 10 c.c. will take 1 minute for injection and the total amount of fluid injected will be 200 c.c. Injection of fluid beyond this limit into a patient whose blood pressure is very low is likely to cause pulmonary œdema which may prove very serious in certain cases of pernicious malaria. A dilution less than that may make it very difficult to inject at the rate of 1/120 gr. of quinine per second.

The danger of injecting saline in large quantities was illustrated, in a case of mine, in a collapsed state suffering from pernicious malaria, in whom an injection of a pint of normal saline was followed by a very marked pulmonary œdema. I cannot, therefore, recommend dilution of 15 gr. of quinine in 4 to 6 pints, as has been recommended by some authorities, which would mean a dilution of 1 in 3000 to 1 in 4000. In my opinion, injection of quinine in strength of 15 gr., dissolved in 5 c.c. given in $1\frac{1}{2}$ to 2 minutes, is dangerous, although considered by others to be a safe procedure. This would mean injecting 1/6 gr. to 1/8 gr. per second and may prove dangerous.

Changes in Blood Pressure

In some cases I made observations on the systolic, diastolic and pulse pressures, and occasionally I found that while the systolic pressure would tend to fall, the diastolic

pressure would tend to rise, making the pulse pressure lower than before, which would be very dangerous to circulation if the systolic pressure is already very low. I have also observed that the more concentrated the solution, the greater is the chance of the pulse pressure being lowered and sometimes there is a marked irregularity in the systolic blood pressure for some seconds after the injection has been given, pointing, as it were, to the conclusion that the systolic output of the heart is irregular for a few seconds after the injection. These changes in blood pressure are all-important factors in the operation of intravenous injection of quinine ; the more concentrated is the solution, the more likely are they to take place.

Regulation of Rate of Injection

It may be argued that a concentrated solution. may be injected very slowly and so there may be no need of diluting the solution. As pointed out before, to inject 10 gr. dissolved in 200 c.c. in 20 minutes would require 1 minute for each 10 c.c. It would, therefore, be necessary to inject the solution still more slowly if a concentrated solution is used, and if it is intended to inject the same quantity of quinine per second. Thus for a solution of 10 gr. in 100 c.c., one must inject 5 c.c. in not less than one minute, and the stronger the solution the lower must be the rate of flow and the more difficult to regulate the rate of flow. Concentrated solution of 20 per cent., or 15 gr. in 5 c.c., as has been recommended by some authorities would mean that one must inject 5 c.c. in 30 minutes. It is evident that this is hardly practicable. For the purpose of regulating the flow very accurately, I have devised a specially fine needle with which the flow can be easily regulated at the rate of 10 c.c. of

saline per minute. I have observed that the use of Bayliss's solution in place of normal saline does not diminish the tendency towards fall of blood pressure after injection of concentrated solution of quinine. From what I have stated above, it is evident that the rate of injection should be still slower in case of children, because if in the adult the limit should be 1/120 gr. of quinine, in the case of children this should be still less. I would suggest that not more than 5 gr. should be injected in patients below 15 years of age in 20 minutes, which is half the rate in the case of an adult.

Two Stages of Fall of Blood Pressure after Intravenous Injection of Quinine.

The fall of blood pressure after intravenous injection of quinine may take place in two stages : (1) A fall that may take place immediately after injection, and (2) a fall that may come on some minutes after injection has been completed and when the quinine has been well diluted in the circulation.

The former may prove rapidly fatal and the latter may happen whatever may be the dilution of the quinine used. To guard against them I would advise that in all cases of malarial fever in which the blood pressure is low—as is frequently the case with the pernicious type of the disease— intravenous injection of quinine should be given guarded with injection of pituitrin or adrenalin. Whatever may be the advantages of a 10 c.c. syringe, and however simple and quick the procedure may be when it is used, I consider that a concentrated solution of quinine should never be rapidly injected intravenously, and as it may be impracticable to inject such a concentrated solution very slowly, a dilute solution of the strength recommended should always be used.

Table showing Results of 5 gr. of Quinine Bihydrochloride dissolved in 100 c.c. of Normal Saline.

	Duration of each injection in seconds.	Quantity in c.c. of fluid injected each time.	Pressure		
			Systolic	Diastolic	Pulse
Before injection	—	—	95	65	30
1	40	10	95	65	30
2	30	10	95	67	28
3	40	10	95	70	25
4	45	10	95	68	27
5	35	10	95	70	25
6	25	10	95	68	27
7	35	10	95	70	25
8	35	10	95	65	30
9	32	10	95	72	23
10	40	10	90	65	25
Minutes after completion of injection		4	92	65	27
		9	90	70	20

The accompanying table shows typical effects on systolic, diastolic and pulse pressures after intravenous injections of dilute solutions of quinine bihydrochloride.   It will be observed that the injections were given more or less slówly. There was still in some cases a tendency towards fall of systolic and rise of diastolic pressures.   It is to be noted that in this paper "solution of quinine" always means solution of quinine bihydrochloride in normal saline.

CONCLUSIONS

1.   In giving intravenous injection of quinine bihydro-chloride the solution of the salt should not exceed the strength

of 1 in 300 ; it should be injected at the rate of 10 c.c. per minute in the case of patients above 15 years of age.

2.   The rate should be half the above in the case of patients below 15 years of age.

Another paper on *The Dangers of Rapid Intravenous Injection on Concentrated Solution of Quinine* was published in July, 1932 in the Journal of Tropical Medicine and Hygiene, the first part of which was similar to what has been quoted above from the Lancet. (Editor.)

The following tables quoted from the last paper give a further series of cases showing the effects on systolic, diastolic and pulse pressures after intravenous injections of dilute solutions of quinine bihydrochlor.   The injections were given more or less slowly.   Still, as noted in the previous paper, in some cases there was a tendency towards fall of systolic and rise of diastolic pressures, but there was never such profound fall of blood pressure as may follow intravenous injection of a concentrated solution of quinine bihydrochloride, as were shown by the author in his previous papers.

Solution of Quinine Bihydrochloride in Normal Saline

10 gr. of Quinine Bihydrochlor. dissolved in 200 c.c. of normal saline

	Duration of each injection.	Quantity of fluid injected each time.	Systolic	Diastolic	Pulse pressure.
Before injection	...	... ...	... 110	... 80	... 30
(1) ...	.. 20 sec. ...	30 c. c.	... 110	... 85	... 25
(2) ...	... 25 ,, ...	30 ,,	... 105	... 85	... 20
(3) ...	... 20 ,, ...	30 ,,	... 104	... 85	... 19
(4) ...	... 15 ,, ...	30 ,,	... 104	... 85	... 19
(5) ...	... 25 ,, ...	30 ,,	... 102	... 85	... 17
(6) ...	... 50 ,, ...	50 ,,	... 104	... 84	... 20
5 minutes after completion of injection ...			... 104	... 80	... 24
15 ,, ,, ,, ,, ...			... 104	... 80	. 24
22 ,, ,, ,, ,, ...			... 104	... 80	... 24
9 hours ,, ,, ,, ...			... 96	... 74	... 22

10 gr. of Quinine Bihydrochlor. dissolved in 200 c.c. of normal saline

		Duration of each injection.	Quantity of fluid injected each time.	Systolic	Diastolic	Pulse pressure.
Before injection	...	... ...	...	... 105	... 83	... 22
(1)	...	... 32 sec. ...	30 c.c.	... 105	... 84	... 21
(2)	...	... 25 ,, ...	30 ,,	... 105	... 85	... 20
(3)	...	... 30 ,, ..	30 ,.	... 104	... 85	... 19
(4)	...	... 25 ,, ...	30 ,,	... 104	... 85	... 19
(5)	...	... 25 ,, ...	30 ,,	... 103	... 86	.. 17
(6)	...	... 35 ,, ...	50 ,,	... 103	... 84	... 19
5 minutes after completion of injection			...	... 103	... 83	... 20
10 ,,	,,	,, ,,	...	... 104	... 86	... 18
14 ,,	,,	,, ,,	...	... 104	... 82	... 22
24 ,,	,,	,, ,,	...	... 103	.. 84	... 19
8½ hours ,,		,, ,,	...	... 99	... 75	... 24

5 gr. of Quinine Bihydrochlor. dissolved in 100 c.c. of normal saline

		Duration of each injection.	Quantity of fluid injected each time.	Systolic	Diastolic	Pulse pressure.
Before injection	...	... ...	...	... 110	.. 75	... 35
(1)	...	... 1 min. ... 40 sec.	30 c.c.	.. 105	... 78	... 27
(2)	...	... 1 min. ... 4 sec.	40 ,,	... 100	... 80	... 20
(3)	...	... 1 min. ... 25 sec.	30 ,,	... 101	... 78	... 23
5 minutes after completion of injection			...	... 105	.. 85	... 20
10 ,,	,,	,, ,,	...	... 105	... 85	... 20
23 ,,	,,	,, ,,	...	... 105	... 84	... 21
30 ,,	,,	,, ,,	...	... 105	... 84	... 21
9 hours	,,	,, ,,	...	... 95	... 75	... 20

5 gr. of Quinine Bihydrochlor. dissolved in 100 c.c. of normal saline

		Duration of each injection.	Quantity of fluid injected each time.	Systolic	Diastolic	Pulse pressure.
Before injection	...	... ...	...	... 115	... 80	... 35
(1)	...	... 50 sec. ...	30 c.c.	... 115	... 85	... 36
(2)	...	... 45 ,, ...	40 ,,	... 110	... 85	... 20
(3)	...	... 65 ,, ...	30 ,.	... 110	... 85	... 25
5 minutes after completion of injection			...	... 110	... 86	.. 24
10 ,,	,,	,, ,,	...	... 110	... 85	... 25
15 ,,	,,	,, ,,	...	... 105	... 85	... 20
20 ,,	,,	,, ,,	...	... 107	... 85	... 22
25 ,,	,,	,, ,,	..	... 105	... 84	... 21
11 hours	,,	,, ,.	...	... 105	... 64	... 41

5 gr. of Quinine Bihydrochlor. dissolved in 100 c.c. of normal saline

	Duration of each injection.	Quantity of fluid injected each time.	Systolic	Diastolic	Pulse pressure.
Before injection	... ...	...	... 95	... 55	... 40
(1) ε ...	... 1 min. ... 45 sec.	55 c.c.	... 92	... 58	... 34
(2) ...	... 2 min. ... 15 sec.	50 ,,	... 95	... 55	... 40
2 minutes after completion of injection	..		... 90	... 60	... 30
5 ,, ,, ,, ,,	...		... 93	... 59	... 34
12 ,, ,, ,, ,,	...		... 92	... 59	... 33
7 hours ,, ,, ,,	...		... 95	... 61	.. 34

5 gr. of Quinine Bihydrochlor. dissolved in 100 c.c. of normal saline

	Duration of each injection.	Quantity of fluid injected each time.	Systolic	Diastolic	Pulse pressure.
Before injection	... ...	...	... 88	... 55	... 33
(1) ...	... 1 min. ... 15 sec.	50 c.c.	.. 88	... 58	... 30
(2) ...	... 1 min. ... 20 sec.	50 ,,	... 85	... 55	... 30
4 minutes after completion of injection	...		... 85	. 58	... 27
7 ,, ,, ,, ,,	...		... 82	... 60	... 22
12 ,, ,, ,, ,,	...		... 82	... 59	... 23
9 hours ,, ,, ,,	...		... 88	... 56	... 32

5 gr. of Quinine Bihydrochlor. dissolved in 100 c.c. of normal saline

	Duration of each injection.	Quantity of fluid injected each time.	Systolic	Diastolic	Pulse pressure.
Before injection	... ...	...	... 95	... 60	... 35
(1) ...	... 1 min. ... 60 sec.	40 c.c.	... 90	.. 60	... 30
(2) ...	... 1 min. ...	40 ,,	... 90	... 63	... 27
(3) ...	... 1 min. ... 5 sec.	20 ,,	... 88	... 60	... 28
5 minutes after completion of injection	...		... 82	... 60	... 22
20 ,, ,, ,, ,,	...		... 85	... 63	... 22
10 hours ,, ,, ,,	...		... 95	... 60	... 35

A PRELIMINARY REPORT ON THE MINIMUM CURATIVE DOSE OF QUININE IN THE TREATMENT OF MALARIAL FEVER BY THE INTRAVENOUS METHOD

The duration of treatment of malarial fever by quinine is still a matter for discussion, and different authorities give different opinions on the subject. Castellani and Chalmers state that their ''routine practice has been to continue with 10 grains three times a day for a month after the cessation of the fever, 5 grains three times a day during the second month, then 5 grains twice a day during the third month.'' In the treatment of malarial fever by the intravenous injection of quinine, a method which one sometimes adopts in cases suffering from recurring attacks, the physician has to decide when there has been complete sterilization of the system. Negative results on examination of the peripheral blood are of no help whatever, as it is a well-known fact that the parasites may remain in the internal organs during treatment with quinine, although the peripheral blood may not show the parasites. The complement-fixation test recently introduced by Gordon Thomson of London School of Tropical Medicine may, when perfected, tell us whether and when a malarial infection has died out; but the test is highly technical, and as yet is subject to serious error; clinical observations have, therefore, to be depended upon. In the following cases are recorded my experiences of the minimum doses required to bring

about complete sterilization, being based on the subsequent history of the patient kept under my observation. Such observations are frequently not free from errors; as re-infection is likely to take place and the only way to be absolutely certain about one's results is to keep the patient in hospital for prolonged periods where the chances of re-infection are slight or non-existent.

It is in this way that one can determine what I have termed *the minimum curative dose of quinine in the treatment of malarial fever by the intravenous method.* In the following cases I have tried to determine this though, in all of them, I could not keep the patients in hospital for indefinite periods.

1. *Nirmala.*—Patient was admitted into my ward on 10-7-19 with a history of recurring attacks of fever coming on every fourth day for three months. The spleen was found enlarged, extending 2″ below the costal margin. The blood showed the presence of quartan parasites.

Treatment.—10 grains of quinine were given intravenously from 10-7-19 for seven successive days.

Result.—Patient free from fever for more than a year. She is still in hospital free from fever. No parasites could be found in the peripheral blood on repeated examination. On 15-8-20 no parasite was found on spleen puncture, *i.e.*, nearly one year after completion of treatment.

Conclusion.—The parasites have been completely destroyed, and patient completely cured.

2. *Ellen.*—Patient was admitted into my ward on 30-1-19. He gave a history of recurring attacks of fever coming on every fourth day. He contracted the disease in Assam and had been suffering for more than six months. The blood showed quartan parasites. Spleen extended 4½″ below the costal arch. (*See* Temp. Chart I.)

Treatment.—(1) 5 grains of quinine given intravenously on 2-2-19, three hours before the expected paroxysm. Fever recurred on 5-2-19.

Chart I
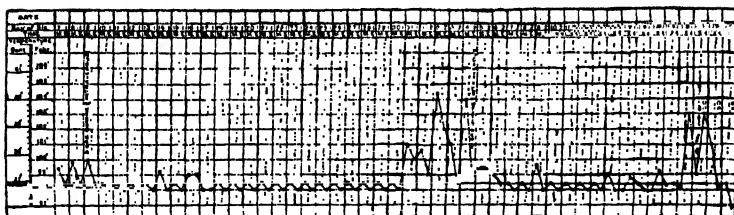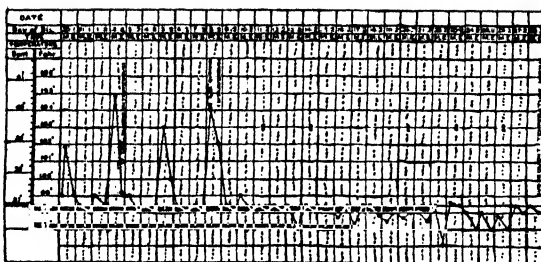
CHART II

Minimum curative dose of quinine

(2) 10 grains of quinine given intravenously on the expected days of the paroxysm and three hours before the expected attack, *i.e.*, on (1) 8-2-19 ; (2) 11-2-19; (3) 14-2-19 ; (4) 17-2-19 ; (5) 20-2-19 ; (6) 23-2-19 ; (7) 26-2-19.

Result.—No recurrence since. Patient remained free from fever for two months in hospital after the second course of treatment and has been reported free from fever since, *i.e.*, for more than a year and-a-half.

Conclusion.—The parasites have been completely destroyed, and patient completely cured.

3. *Annoda.*—Patient was admitted into my ward on 6-1-19. He gave a history of recurring attacks of fever for three months. Blood showed the presence of benign tertian parasites. Spleen extended 2″ below the costal arch. (*See* Temp. Chart II.)

(1) 5 grains of quinine given intravenously on 9-1-19.

Fever recurred on 2-2-19 (parasites in blood).

(2) 10 grains of quinine given intravenously on˙ 4-2-19.

Fever recurred on 12-2-19 (parasites found).

(3) 10 grains of quinine given intravenously on 20-2-19 and three consecutive days.

Fever recurred on 13-3-19 (parasites found).

(4) 10 grains of quinine given intravenously on 14-3-19 and four consecutive days.

Fever recurred on 5-4-19 (parasites found).

(5) 10 grains of quinine given intravenously on 7-4-19 and six consecutive days.

Result.—No recurrence after the last treatment with quinine. Patient remained in hospital for 6 months since the last injection.

Conclusion.—The parasites have been completely destroyed, and patient completely cured.

4. *Kangli.*—Patient was admitted into my ward on 24-3-19. He gave a history of recurring attacks of fever for four months. Blood showed the presence of benign

tertian parasites.   Spleen extended 2″ below the costal arch.
(*See* Temp. Chart III.)

(1) 10 grains of quinine given intravenously on 15-3-19.

Fever persistent (parasites present).

(2) 10 grains on 18-3-19 for five consecutive days.

Fever persistent (parasites present, but very few).

(3) 10 grains from 29-3-19 for seven consecutive days.

Patient free from fever since the last injection.   Patient
remained in hospital for two months free from fever, and
has reported since to have had no recurrence up to now,
which is nearly a year and-a-half after the treatment was
concluded.

Conclusion.—The parasites have been completely
destroyed, and patient completely cured.

5.   *Khirshed.*—Patient was admitted into my ward on
26-4-19.   The blood showed the presence of benign
tertian parasites.   Spleen extended 2″ below the costal arch.
He gave a history of recurring attacks of fever for two
months.

(1) 10 grains of quinine given intravenously on 5-5-19
and on 10-5-19.

Fever recurred on 20-5-19 (presence of parasites).

(2) 10 grains of quinine given intravenously from 20-5-19
for seven consecutive days.

Result.—Patient free from fever since the last injection.
Was in hospital for nearly two months after being free
from fever.

No conclusion can be drawn from the case, as patient
was under observation for only two months.

6.   *Purna.*—Patient was admitted into my ward   on
19-6-19.   The blood showed the presence of benign tertian
parasites.   He gave history of recurring attacks of fever.
(*See* Temp. Chart IV.)

(1) 6 grains of quinine given intravenously from
25-6-19 for three consecutive days.
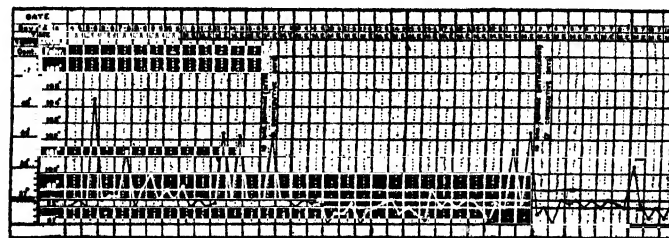
Minimum curative dose of quinine

Fever recurred on 14-7-19 (*parasites present*).

(2) 10 grains of quinine given from 16-7-19 for seven consecutive days.

Result.—Patient remained free from fever since the last injection.

No recurrence for six months, after which patient could not be traced.

Conclusion.—Parasites completely destroyed, and patient completely cured.

7. *Maditar.*—Patient was admitted into my ward on 3-6-19. He gave a history of recurring attacks of fever. Blood showed the presence of benign tertian parasites. Spleen extended 2″ below the costal arch.

(1) 10 grains of quinine given intravenously from 16-6-19 for five consecutive days.

Fever recurred on 2-7-20. (*See* Temp. Chart V.)

(2) 10 grains of quinine given intravenously on 3-7-20 for seven consecutive days.

Result.—Patient had no fever since the last injection. He was in hospital for nearly three months free from fever.

In all the above cases, no parasites could be detected on repeated examination of the blood after the treatment was completed.

8. *Laha Singh.*—Patient was admitted into my ward on 15-6-19. Blood showed the presence of malignant tertian parasites with crescents.

(1) 10 grains of quinine given on 22-6-19 and 23-6-19.

Fever relapsed on 3-7-19.

(2) 10 grains of quinine given from 3-7-19 for seven consecutive days.

Result—The patient had no fever since the last injection. He was in hospital for nearly two months after the last injection during which time he was free from fever. Subsequent history of the patient could not be traced.

From the above cases the following conclusions may be drawn :—

(1) In recurring benign tertian infections, 10 grains of quinine must be given intravenously for, at least, seven successive days to bring about complete sterilization.

(2) In recurring quartan infections, 10 grains of quinine must be given intravenously for, at least, seven days to bring about complete sterilization. In one case complete sterilization was brought about by giving the injections on the expected days of the paroxysm, and in another case by giving the injections for seven successive days.

(3) The reason why several intravenous injections of quinine have to be given to bring about complete sterilization can be explained by the fact that quinine is quickly eliminated by the kidneys after intravenous injection and the whole of it may be excreted before all the parasites have been destroyed.

I cannot agree with Col. A. G. Phear, who, in a lecture at a meeting of the Royal Society of Medicine held in March, 1920, stated that it was impossible to eradicate the malarial parasite from the system in cases of relapsing malaria by any known method of treatment, including the administration of quinine.

It will be seen from the above that though my observations are limited, yet so far they differ from those of Stephens and his colleagues, who consider that intravenous injections of quinine effect only a temporary cure of simple tertian malarial fever and cannot prevent relapses. It is, however, quite possible, as has been observed by James, that in some cases intravenous injection of quinine may fail to prevent the occurrence of relapses, and such cases will form the subject of future investigations. I would also point out that, according to some, it is possible that in the blood vascular system there are regions that are consistently free from quinine throughout a period of quinine treatment,

DIFFICULTIES IN THE DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS OF MALARIAL FEVER*

In a recent text-book with which most of you are familiar the following remarks have been made. " The diagnosis of malaria may be simplicity itself or on the other hand it may be most difficult as there is practically no sign or symptom of disease of the human body which it cannot mimic." I am afraid I may have to traverse the whole field of medicine in a discussion on the difficulties in the diagnosis and differential diagnosis of malarial fevers which may be impossible to complete within the time at my disposal. You cannot, therefore, consider my paper as an exhaustive study of what is stated in its title.

It may, on the one hand, simulate some of the rarest of diseases such as icterus gravis or, on the other hand, it may exhibit such a diversity of symptoms as to simulate an acute abdomen or a cerebral abscess.

The difficulty in the diagnosis of the disease may be considered under the following heads :—

(1) Cases that are diagnosed as malaria when it does not exist.

(2) Cases that are not diagnosed as malaria when it really exists.

In the diagnosis of a tropical fever, the possibility of malaria should always be remembered, but as multiple

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infections are not uncommon, never accept malaria as a complete diagnosis till other conditions have been excluded. Nor must it be forgotten that most of the fevers of cool climate are prevalent in the tropics and, therefore, they must be taken into consideration while studying a case of fever in the tropics.

Early tuberculosis is likely to be mistaken for malaria. The disease may be associated with rigors, the temperature may be of an intermittent nature rising to 103°F or 104°F. Together with this there may be slight enlargement of the spleen and cough may not be much complained of. Slight leucopenia may be present in both diseases. In the early stages, the physical signs pointing to the lungs may be very slight. On the other hand, insufficient doses of quinine to a case of malaria may make the parasites disappear from the peripheral circulation and if the fever is persistent, one may think that the fever is due to tuberculosis while it may be nothing but an improperly treated case of malaria.

I shall briefly refer here to some of the early symptoms of tuberculosis which should be of help in its diagnosis. The apices of the lungs should be carefully examined, examination should be made by means of X-rays in obscure cases whenever possible, especially of the hilus or of the infraclavicular triangle of Schut. Examine also for Reviere's bands of impairment, the presence of paravertebral dulness, and the contraction of Krönig's isthmus. Examine the sputum for tubercle bacillus, for albumin reaction and for its cytological character. In obscure cases inoculation experiments for the development of tuberculosis in a susceptible animal should be performed. On the other hand, the blood should be examined carefully for malarial parasites, for pigmented leucocytes or increase of mononuclear leucocytes. In all suspected cases the therapeutic test by administration of quinine should also be carried out. The diagnosis may be extremely difficult in cases in which the lesions in the

lungs are slight and the chief seat of lesion is in the intestines or the kidneys. I shall describe here one such case : A patient came under my observation for treatment of fever of an intermittent nature and, with enlargement of the spleen. There was slight leucopenia. The history of profuse sweats at night, the failure of the quinine test for malaria and the negative results obtained from examination of the splenic blood and culture of the peripheral blood for leishmania led me to examine the stools for tubercle bacilli in which they were present in fair numbers. The patient subsequently developed unmistakable signs of pulmonary tuberculosis.

Other lung conditions such as bronchitis or pneumonia or pleurisy may be only manifestations of malaria. In cases of pneumonia occurring as a manifestation of malaria, the fever may be of an intermittent type. On the other hand, pneumonia may complicate malaria and, therefore, the initial symptoms may be somewhat benefited by administration of quinine, the fever subsequently not being benefited by it.

Liver abscess is not infrequently mistaken for malaria and for one who has to deal with fevers in the tropics, this is a very important fact to remember. Let us discuss the diagnosis of the following types of cases : Patient gives history of a previous mild attack of dysentery or diarrhoea ; there is history of fever of an intermittent nature coming on at irregular intervals with rather long apyrexial periods ; the spleen is not enlarged and there is some amount of hepatic tenderness below the costal margin ; there is a slight leucocytosis with slight polymorphonuclear increase or lymphocytosis. Such a case is frequently mistaken for malaria and treated with massive doses of quinine. I can recall here a case in which for several weeks the patient was treated with quinine till an exploratory puncture of the liver revealed the true nature of the disease. The patient,

æt. 25, gave an indefinite history of dysentery sometime previously. He came under my treatment suffering from attacks of fever of an intermittent nature followed by profuse sweats and pain in the right shoulder joint. There was no enlargement of the spleen and no malarial parasites were present in the, blood, which was attributed to the previous administration of quinine to the patient. The fever was so typical of malarial attacks that one was not justified in withholding exhibition of large doses of quinine which were subsequently given intravenously. There was an apparent improvement and the patient was free from fever for a few weeks but he again began to suffer from fever coming on twice and sometimes thrice during 24 hours and followed by profuse sweats. The liver was distinctly enlarged upwards and friction sounds were audible at the base of the right lung. A tender spot was discovered in the 5th intercostal space in the midaxillary line and an exploratory puncture revealed the presence of pus. One must, therefore, make a very careful examination of the liver in cases with hepatic enlargement in which quinine is not followed by any benefit. Generally speaking, there is leucocytosis in these cases and careful examination will reveal local tenderness or deep fluctuation in the liver. An X-ray examination may be of help in diagnosis in such cases.

Another condition that is likely to be mistaken for malarial fever is *hepatic intermittent fever* of Charcot. All of us are familiar with a condition of the following nature :— Patient comes with a history of fever coming on with shivering and colicky pain in the epigastric or right hypochondriac region. There is tenderness below the ribs on the right side. There is history of intermittent attacks of jaundice and intermittent attacks of colic. Unless properly examined such a case is likely to be mistaken for malarial fever. The history of repeated attacks of colic shooting

towards the right shoulder or backwards towards the inferior angle of the right scapula, the history of pain coming on soon after food, and the history of intermittent attacks of jaundice are all important factors which should be carefully enquired into. The presence of an enlarged gall bladder presenting a pyriform mass in the right hypochondriac region with an enlarged Reidel's lobe, the history of intermittent attacks of jaundice and of colic, and the history of long-standing symptoms referred to as dyspepsia and hyperchlorhydria are factors in the diagnosis of the disease. X-rays may be of help, but not always so.

Other conditions of the liver that may give rise to fever resembling that of malaria are (1) *suppurative cholecystitis*, (2) *malignant disease of the liver* and (3) sudden attacks of high fever that are sometimes met with in cases of *Hanot's cirrhosis*. *Presuppurative hepatitis* may also give rise to fever which may resemble malaria. Weil's disease or a case of catarrhal jaundice may even be mistaken for malarial fever. I have notes of a case of *malignant disease of the liver* in which the symptoms resembled very much those of malaria. The patient came to Calcutta with history of fever of an intermittent nature and jaundice. The spleen was slightly enlarged and the liver could be felt on very careful palpation. He got no benefit from quinine. The jaundice deepened and the liver became more and more enlarged with almost stony hardness. Subsequently the patient died with all the symptoms of cancer of the liver.

Ulcerative endocarditis may be mistaken for malarial fever. I recall here the notes of a case which was once set in a University examination in Medicine. The patient, æt. about 45, gave history of attacks of fever coming on every day with shivering. The spleen was slightly enlarged. He was treated with large doses of quinine but without any benefit. The blood showed absence of malarial parasites, which fact was of no value after so much

administration of quinine. There was marked leucocytosis. I doubted the malarial nature of the disease. The heart revealed the presence of a diastolic bruit at the base, but this was attributed to an *old valvular disease. But in a few days other bruits developed at the apex and a systolic bruit also developed at the base. The bruits also changed their characters. Subsequently the patient began to suffer from fever coming on with rigors, two or even three a day, but there was no regularity in the paroxysms. The patient at last developed embolic symptoms as shown by hemiplegia, hæmatemesis and melæna. There were signs of arthritis and he died. It was no doubt a case of ulcerative endocarditis engrafted upon an old aortic disease. The blood culture was negative and, therefore, confused the diagnosis. It should be remembered here that in ulcerative endocarditis the fever may sometimes even assume a tertian type causing extreme confusion in diagnosis. A careful examination of blood for leucocytosis, absence of pigmented leucocytes and absence of mononuclear-leucocytosis, marked retardations and anticipations in the attacks of fever, the presence of bruits variable in position and character from day to day, and the presence of embolic phenomena were all important factors in diagnosis. The presence of malarial parasites in the blood may not exclude the possibility of ulcerative endocarditis.

Among diseases of the kidneys, *pyelitis* may be mistaken for malarial fever. Intermittent fever associated with rigors is usually present in cases of suppurative pyelitis. The chills may recur at regular intervals and may be mistaken for those of malaria. Examination of the urine and careful study of symptoms referable to the kidneys, leucocytosis and absence of changes in blood that one expects to find in malaria are diagnostic points. It must be remembered that one examination of the urine may not show the presence of pus especially when one kidney is involved and the ureter temporarily blocked,

Malaria may sometimes present symptoms like those of *acute abdomen* and may thus be mistaken for (1) hæmorrhagic pancreatitis, (2) appendicitis or (3) perforative peritonitis.

Cases of subtertian malaria sometimes begin with sudden attacks of violent pain in the epigastrium followed by vomiting and collapse. There may be tympanitis and tenderness in the epigastrium. These cases may be mistaken for acute hæmorrhagic pancreatitis. In suspicious cases, the blood should be examined and if parasites are present the diagnosis is clear. Other cases may begin with colicky pain starting from the region of the appendix and these may be mistaken for appendicitis.

Hodgkin's disease may be passed for malaria when the spleen and the deep-seated lymph nodules are only enlarged and pyrexia with rigor is a prominent symptom constituting what is known as the Pel-Ebstein type of the disease. Examination of the blood for malarial parasites and the therapeutic test with quinine are important factors in the diagnosis of such cases.

Kala-azar is another disease which may be mistaken for malaria. The two diseases may be mistaken for each other in two conditions—(1) acute stage and (2) stage of cachexia. kala-azar sometimes begins with malaria-like attacks of fever coming on with shivering. The most characteristic temperature chart of kala-azar is the double rise during 24 hours and this is present even when the temperature is of an intermittent type in which there may be double rise with double intermission during 24 hours or double rise with single intermission during 24 hours. Double rise of temperature may sometimes, though rarely, occur in malignant tertian infection. There may be an initial rise, a pseudo-crisis, a pre-critical elevation and a crisis, *i.e.*, there may be double rise with a drop of some degrees of temperature during 24 hours. Theoretically speaking, there may be also double rise of

temperature during 24 hours in some cases of double infection with benign tertian parasites in which two sets of parasites may sporulate in different periods of the same day. In such a case there shoud be one complete period of apyrexia on alternate days. If, however, one set of parasites sporulates towards the end of the paroxysm of fever caused by another set of parasites, the fever may continue for some part of the day in which no maturation of parasites takes place and thus may stimulate the fever of kala-azar. On careful examination, the significance of these temperature charts may be made out and the disease properly diagnosed.

Another characteristic feature in the temperature chart of kala-azar is its variable nature, i.e., an intermittent fever may pass on to remittent pyrexia of double quotidian type, which again may pass back to an intermittent type and so on.

I shall not, however, pursue any further the points of differential diagnosis of malaria and kala-azar but would conclude by saying that a case of fever with symptoms resembling those of malaria which has resisted, for a week, quinine given orally in 10 to 15 grain doses per day, inspite of purging by mercury and salines from time to time, and which has afterwards resisted intramuscular or intravenous injection of quinine in 10 grain doses for another week is not malarial and must lead to the suspicion of other diseases among which is to be included kala-azar in an endemic area.

The above procedure of arriving at a conclusion for excluding malaria takes some time and during such a period the case may remain a doubtful one. Fortunately such cases are extremely rare and generally we find that malarial fever yields to quinine in seven days. In these cases the peripheral blood should be cultured for flagellates,

The two diseases are also likely to be mistaken for each other in the late stages :—There are some cases in which the diagnosis from chronic malaria may be very difficult. The differential leucocyte count may show diminution of the polymorphonuclears in both the diseases, while the character-istic leucopenia of kala-azar may be absent in a particular case. Not frequently do we meet with cases with the following blood picture :—RBC—2,500,000, WBC—3,500, Hb—30%. Such a blood picture fits in with chronic malaria just as it does with kala-azar and may present ex-treme difficulties in diagnosis. These cases which may be termed borderland cases between kala-azar and malaria, so far as the examination of the blood is concerned, are not uncommonly met with and are extremely difficult to diagnose. The diagnosis becomes still more difficult, because one hesi-tates to puncture the spleen in such cases as they are generally cachectic and are likely to bleed easily, there being marked diminution in the coagulability of the blood. In such cases spleen puncture may be negative at the first examination and may require to be performed more than once before one is certain of the absence of L. D. bodies in the spleen. Besides, it has been noted that in undoubted cases of kala-azar sometimes no L. D. bodies may be found when smears are made from puncture of the spleen or liver, though their presence may be found by means of cultures or by inoculation into a susceptible animal. Therefore, the absence of L. D. bodies or malarial parasites in a smear from the spleen does not exclude kala-azar or malaria. Such cases with negative results from examination of the smears from the spleen blood are not uncommonly met with and present extreme difficulties in diagnosis.

Here again the blood should be cultured for flagellates. The therapeutic test for malaria should be made and the various serum tests tried. In many cases, the temperature chart, as has been pointed out by James, will bring out the

characteristic curve of the type of malaria and help in the diagnosis. The presence of malarial parasites in the peripheral blood will clinch the diagnosis of malarial fever. Unfortunately, however, in many cases of chronic malaria the parasites may not be detected, at any rate, at one examination of the blood.

It may be stated, in general, that fevers with splenomegaly are all likely to be mistaken for malarial fever.

Malta fever is likely to be mistaken for malaria in places where the two diseases occur. The symptoms of the malignant cases may resemble those of pernicious malaria as shown by hyperpyrexia, heart failure or lung engorgement. The resemblance sometimes may be so very great that it is no exaggeration to state that nearly every case of Malta fever has in its early stages been treated for malaria as has been very aptly pointed out by Basset Smith. The points of diagnosis are (1) examination of the blood for malarial parasites, (2) hæmo-culture for micrococcus melitensis, (3) the agglutination test for Malta fever and (4) the therapeutic test for malaria.

The multitudinous manifestations of pernicious malaria may be mistaken for various acute conditions and may be missed unless one thinks of their possibility. Consider the following case which I once had occasion to meet : Patient, a fat person, aged about 50, was suffering from fever of about seven days' duration. Pulse was 140 and of low tension. He was dyspnœic, very drowsy and his eyes were blood-shot. The tongue was red and dry. There were low muttering delirium and presence of rales and rhonchi at the bases of the lungs. Opinions were expressed by some that the case was one of plague, others thought it to be one of influenzal broncho-pneumonia and some thought that it was typhoid. The possibility of the case being one of pernicious malaria was not suggested by any previous consultant. Enquiring into the history of the case, I was informed that the patient had gone to an intensely malarial place some few

days back, and that though he was supposed to have been suffering from fever for several days, yet it intermitted one day when he was given an insufficient dose of quinine. This at once gave a clue to the real condition of the case. Examination of the blood showed a well marked leucocytosis (30,000) with the presence of a few malignant tertian rings and pigmented leucocytes. The leucocytosis was misleading and evidently terminal. I put the patient on vigorous treatment with quinine consisting of 10 grains every three hours, combined with intramuscular injection of 10 grains of quinine every day. On the first day he had 30 grains by the mouth and 10 grains intramuscularly. The fever intermitted after 72 hours and a very valuable life was saved.

Another type of cases which present extreme difficulty in diagnosis is the *choleraic or the algid type* of malaria. Here again I shall relate to you a case that I met with in consultation with a colleague. A careful observer as he was, he made an immediate examination of the blood but no parasites were found. There was history of severe vomiting and purging followed by collapse. The stools were not typically rice-water and there was not complete anuria. On enquiring into the history of the case, we were informed that the patient had a typical attack of ague about three days back which was followed by gastro-intestinal symptoms and collapse. In this case there was very great difficulty in the diagnosis of the case as no malarial parasites were found in the blood. The spleen was slightly enlarged. The history of the typical attacks of ague fits was so significant that the possibility of malaria could not be excluded from my mind. We gave the patient an intravenous injection of a dilute solution of quinine bihydrochloride (5 grains in 100 c. c) with adrenalin and this was repeated after three hours. The results were nothing short of a magic and the patient recovered. A similar case was recently admitted into my ward and the patient is convalescent.

Pernicious malaria may be mistaken for *cerebral hæmorrhage*. Last year I met with a case of this nature. The history was that the patient who was about 35 years of age became suddenly unconscious after a fit of mental excitement. When I came to see the patient three hours after the so-called stroke, the following conditions were noticed : Temperature about 101°F, no high tension in the pulse, no signs of paralysis and the patient was very stuporous. The spleen was slightly enlarged. On further enquiry, I was told that the patient was suffering from fever for about a week which was of an intermittent nature but not very high and the patient came from a place where there was intense malaria at the time. It was 10 o'clock at night but still I insisted upon the blood being examined at once. This led to the remarkable discovery of the presence of malignant tertian parasites in the blood. Full doses of quinine saved the patient's life.

Another case presented still greater difficulty in diagnosis. The patient, a young boy, seven years old, was seen by me in consultation with a colleague. His eyes were red, neck stiff, Kernig's sign present, the boy was comatose and having convulsions. There was previous history of a sore throat. Though the possibility of *cerebrospinal meningitis* was paramount in my mind, yet I could not give up the idea of the symptoms being of malarial origin. The blood was examined and malarial parasites were found and recovery took place under administration of full doses of quinine.

The possibility of malignant tertian fever resembling many of the specific fevers in its symptomatology has to be remembered. I have already stated that how frequently Malta fever is mistaken for malaria. To attempt to make a differential diagnosis between malaria and other specific fevers which it may resemble, is too vast a subject to dwell upon here. I shall therefore only discuss a few of them here.

(1) *Typhoid*—Sometimes the resemblance to typhoid is very great and there is no doubt that this fact has given rise to the terminology of *typho-malarial* fever.

On the other hand, typhoid fever may take an intermittent course for a few days before it assumes a remittent type—a temperature curve also frequently noticed in malignant tertian infection. Besides, towards the end of typhoid the temperature frequently assumes an intermittent character, giving rise to the curve of steep rises and thus be mistaken for malaria. Clinically it appears to me that if there is a marked early anæmia or an icteroid tinge in the conjunctiva, then the disease is more in favour of malaria. Among other diagnostic signs of typhoid are (1) shortness of hearing, (2) epistaxis and (3) a slow and dicrotic pulse. Marris's atropine test may be positive. In the case of malignant tertian fever presenting a temperature chart like that of typhoid, I have frequently observed that there is a tendency towards intermission after five or six days after the commencement of the illness which is not noticeable in case of typhoid. In suspicious cases and where it is possible the blood should be cultured for typhoid bacillus within the first three or four days of fever and the urine antigen test or the typhoid reaction of Stevens and others tried. Attempts should be made to culture the micro-organism from the urine and the Widal reaction for typhoid should also be looked for. On the other hand, the blood should be examined for malarial parasites, pigmented leucocytes and increase of large mononuclear leucocytes. The therapeutic test with quinine has to be tried in suspicious cases, and in many cases it is better to err on the side of safety by administration of quinine than by withholding it. It may be stated that while in most cases there should be no difficulty in diagnosis, there are others in which in malignant tertian infection, there may be no chills, remissions very slight, there is presence of furred and white tongue, flushed cheeks and

even the' blood examination may not show any parasites for some days. In such cases, if malaria is suspected the blood should be examined more than once at frequent intervals and parasites may be discovered. In hæmorrhagic type of malaria, there may be hæmorrhage from the bowels leading to a mistaken diagnosis of typhoid fever. I present to you the signs and symptoms of a difficult case for diagnosis : Patient gave history of malaise for a few days before commencement of illness. There were chills at the beginning with tendency towards intermission of the fever which subsequently took a continuous course. There was slight anæmia, pulse was 110 and temperature was 102°F. Sordes in the lips, low muttering delirium, slightly enlarged spleen, tumid abdomen, and mild gastro-intestinal symptoms were present. At first five grains of quinine given three times for one day failed to stop the fever. There were melæna and hæmaturia on the 7th day of illness shortly after administration of quinine and the attending physician thought that they were due to quinine. Subsequently the fever responded to quinine and the patient was cured. Such a case, no doubt, gives a clinical picture very much resembling typhoid but if there is any doubt of malaria, give quinine in sufficient doses. In such a case if quinine is withheld there may be a desperate stage when quinine will be of no use especially in cases of malignant tertian infection.

The diagnosis from *paratyphoid* infection will have to be made on the same lines as that from typhoid.

(2) *Typhus*—Sometimes malarial fever resembles *typhus* and this fact has to be borne in mind.

(3) *Cerebrospinal meningitis* is sometimes mistaken for malarial fever. Confusion is specially likely to take place in those cases of cerebrospinal fever in which the fever is of an intermittent type. In a case presenting the classical symptoms of cerebrospinal meningitis with leucocytosis, one should not be satisfied by assuming that the case is not one of

malarial fever. In such cases lumber puncture should be made and a cytological examination made of the cerebrospinal fluid. Recently I had a case which was at first considered to be one of cerebral malaria but the cerebrospinal fluid showed a leucocytosis of 40,000 corpuscles per c. mm. in which the polynuclears were 85%, the fluid was turbid and specific treatment with antimeningococcic serum together with intravenous injection of acriflavine cured the patient. On the other hand, severe headache, Kernig's sign, stiffness of the neck, and coma may be present in pernicious malaria. In such a case, the spleen is generally enlarged and blood shows the presence of malarial parasites, the cerebrospinal fluid is generally clear and there is absence of the specific micro-organism of cerebrospinal meningitis in the fluid. We may meet with the following type of cases and be tempted to diagnose the case as one of malaria or cerebro-spinal meningitis complicated with malaria : A patient is brought in a comatose condition with marked Kerhig's sign and stiffness of the neck. The fever subsides but after some hours it again rises to 103° or 104° with a rigor. In such cases the blood should be examined for malarial parasites and also for leucocytosis with polynuclear increase. I consider that if there is the slightest suspicion of malaria as shown by absence of leucocytosis with mononuclear increase and presence of an enlarged spleen, such a case should be treated with quinine and a lumber puncture made for purpose of diagnosis. If no specific micro-organism is found, and there is no increase of leucocytes and the fluid is perfectly clear, one should not hesitate to treat the case with quinine for some time if there is any suspicion of malaria. One should also remember that rise of temperature with rigors is not uncommon in cerebrospinal meningitis especially after intrathecal injection of antimeningococcic serum.

(4) *Influenza* is another disease which may be mistaken for malaria. More important is the fact that many cases of

malaria are mistaken for influenza especially during an epidemic of the latter disease and there is no doubt that many fatalities occurred during the last epidemic of influenza and that the most important factor in determining their prognosis was complicating malaria.

Consider the following case that I am quoting.—Paroxysms with temperature rising to 105°F or 106°F with remission to about 101°F. Maniacal symptoms, delirium and jaundice were present. Quinine was administered by mouth for three days. Temperature came down to 99°F—102°F. There was no delirium now, but profound anæmia was present. On the 12th day, 7 grains of quinine were given intravenously and repeated next day. Temperature fell to normal on the evening of the 13th day and convalescence proceeded normally with only two mild relapses. The case is no doubt a difficult one, but the diagnosis was made clear by the discovery of malarial parasites in the blood.

There is no doubt that many of those cases that were met with in a comatose condition during the last epidemic of influenza and treated as influenza were cases of cerebral malaria or influenza complicated with malaria.

(5) *Plague* may be mistaken for malaria and *vice versa.* I have already mentioned to you a case of pernicious malaria which was mistaken for plague. Similarly plague may be mistaken for malaria. I remember a case admitted into my ward in the Medical College when I was a House Physician, with history of fever coming on with very severe shivering, temperature rising to 107°F. The spleen was enlarged and there was history of a previous attack of intermittent fever about seven days previously and apparently cured by quinine. The patient was in the general ward for two days. His blood was examined for malarial parasites with positive findings but along with this there was a very marked leucocytosis which could not be accounted for. Towards the evening of the second day, the patient developed

a bubo in one of the groins and was transferred to what used to be called in those days the infectious ward in the Medical College and the patient died. Typical plague bacilli were found in the materials from gland puncture. It was evidently a mixed infection of malaria and plague. It is evident that the differential diagnosis from pernicious malaria may be impossible without a complete bacteriological examination of the blood. The possibility of making mistakes between malaria and plague where the disease has been recently introduced should not be forgotten.

(6) *Relapsing fever*—This is frequently mistaken for malaria in areas where both diseases occur. I remember a case that came from Delhi some years ago and was admitted into the Campbell Hospital. There was jaundice and the spleen was enlarged. I was treating the case as one of malarial fever and the apparent coincidence of a crisis with administration of quinine led me to consider the diagnosis as correct. There was another attack and temperature remained high for a few days. The blood was examined for malarial parasites but showed the presence of the spirochætæ. Enquiring into the history of the case it was discovered that the patient came from Delhi. I wrote a letter to Sir Pardey Lukis who was then in Delhi and he wrote to say that quite recently a number of cases of relapsing fever had occurred in Delhi. The patient was treated with salvarsan and was cured. Of course if the temperature is that of a fever remaining remittent for three or four days and then followed by crisis and then after a period of quiesence another attack takes place, the possibility of this disease should not be forgotten and the blood should be examined for the spirochætæ and agglutination reaction.

(7) Cases of *subtertian infection* may sometimes begin with attacks of pain in the appendicular region with vomiting. There may or may not be rigors. The case may be treated as one of *appendicitis* while it may be only malaria.

(8) Sometimes pernicious malarial fever begins with marked *dysenteric* symptoms. There may be typical dysenteric motions containing blood, muco-pus or there may be hæmorrhagic motion without pus and with little or no mucus. High fever with great distress and prostration and a small rapid pulse may be present. The case may be treated for dysentery while the only treatment is specific treatment with quinine.

(9) I have already stated that malarial fever may be mistaken for *cholera* and cases of pernicious malaria are occasionally admitted into the cholera ward of a hospital due to a mistake in diagnosis.

(10) I have already mentioned that malaria may simulate various conditions that give rise to *acute abdomen*, such as, *hæmorrhagic pancreatitis* or *acute cholecystitis* or *acute peritonitis*. In suspected cases the possibility of malaria should be always borne in mind.

(11) You are all aware that *filarial* fever may sometimes be mistaken for malaria.

(12) *Syphilis* may sometimes be diagnosed as malarial fever. Internal syphilis may give rise to fever resembling that of malaria. Consider the following case : Patient gave history of intermittent attacks of fever from which he was suffering for about two months. Physical examination of the lungs revealed signs of old pleurisy in the right side of the chest. No malarial parasites were present in the blood but the nature of the fever justified the continuance of the quinine which did not, however, stop the fever. The patient was seen by several consultants and the diagnosis of tuberculosis was in the minds of most of them. A full course of treatment with mercury and intravenous injection of salvarsan cured the patient. In another case which was being treated with massive doses of quinine, the diagnosis was made by the accidental discovery of an ulcer in the leg which looked like a typical broken

down gumma. Wasserman reaction was not performed but the exhibition of full doses of mercury with iodide of potassium completely cured the patient.

(13) *Coli infection* may be the cause of fever resembling that of malaria. Generally speaking there is leucocytosis and there may be more than one rise of temperature' during 24 hours.

(14) Malaria may give rise to symptoms which may simulate those of *icterus gravis*. Consider a case : It began with fever associated with jaundice, bilious vomiting and diarrhœa. Pain and tenderness over the hepatic region. The fever persisted and there was a remission in the symptoms but the patient got hiccough, epistaxis, and hæmatemesis and the temperature rose again. He became comatose and died. Such a case is likely to be mistaken for acute yellow atrophy of the liver. A case like this was recently admitted into my ward.

(15) Consider again another type of cases that are likely to lead to a mistaken diagnosis : A patient is brought into hospital with irregular fever, severe epigastric pain, bilious vomiting—the vomit containing blood, loose hæmorrhagic stools and hæmatemesis. He suffers from severe pain over the body. There are hæmorrhages under the skin. Finally he gets an attack of hæmoptysis and suddenly becomes dyspnœic and dies. Cases of this type may be mistaken for *hæmorrhagic small-pox*.

I now pass on to another series of cases that are likely to be mistaken for malaria and these are the various conditions that give rise to coma. Consider a case brought into hospital with intense headache and high rise of temperature. This is quickly followed by profound coma and death. Such a case may be mistaken for *hæmorrhage into the pons or crus cerebri*. Consider another case admitted into hospital in a semicomatose condition with *epileptiform fits*. Such a case may also be mistaken for

cerebral hæmorrhage. Cases may even manifest localizing symptoms such as *hemiplegia and hemianæsthesia.*

Diabetic coma may be mistaken for malaria. I mention here the history and signs and symptoms of a case which I observed sometime ago. He was diabetic and also had large amount of albumin in the urine. He came to Calcutta for a few days and was intending to leave for Dacca when one morning he came in for high fever with rigor. We suspected malaria and before the blood could be examined we gave him full dose of quinine. The fever came down but rose again the next day with shivering and the patient became comatose. No malarial parasites were found, the urine showed the presence of acetone bodies. We gave him an intramuscular injection of quinine. The fever intermitted and he regained his consciousness but towards the evening the fever again appeared, the patient passed into a comatose state, there was marked diminution in the quantity of urine, dyspnœa supervened and the patient died.

Malaria may present symptoms like those of *heat-stroke* and it may be stated that in a doubtful case of heat-stroke inject quinine as the case may be one of cerebral malaria.

Cases are on record which have been mistaken for *cerebral abscess.*

Another type of cases that are likely to give rise to mistaken diagnosis are cases that die suddenly with symptoms of syncope or of acute progressive anæmia or an acutely developing œdema with nephritis and albuminuria. Such cases if diagnosed and treated with proper doses of quinine may recover as was the case with a servant of mine in Dacca.

I shall not enter into a discussion of the various conditions that give rise to various manifestations of what is known as *latent malaria* nor the various nervous manifestations including the psychosis that may be manifestations

of malarial infection. I shall only mention to you one case which resembled the symptoms of *disseminated sclerosis* in a remarkable way : Patient came into hospital with history of attacks of intermittent fever and enlargement of the spleen. The patient had scanning speech and intentional tremors and his knee-jerks were increased. In the hospital he had an attack of fever with rigors and those symptoms increased. Blood was examined and showed the presence of quartan parasites. Administration of full doses of quinine completely cured all the symptoms.

I shall conclude my observations by giving a list of diseases, modified from a recent *System of Tropical Medicine*, which may be mistaken for malaria :—

Anæmia and debility, aphasia, appendicitis, bronchitis, cerebrospinal meningitis, coma, cerebral hæmorrhage, convulsions, cholera, disordered action of heart, dysentery, epilepsy, hæmorrhagic pancreatitis, influenza, icterus gravis, kala-azar, Malta fever, neuralgia and neurasthenia, plague, rheumatic fever, sandfly fever, simple continued fever, trench fever, paratyphoid fever, typhoid fever, typhus, septic endocarditis, yellow fever, worms, acute peritonitis, cerebral abscess, polyneuritis, various forms of psychosis, hydrophobia, tetanus, convulsions in children, neuritis, bulbar paralysis, and diseases of the ductless glands.

Let me end the subject of my lecture by noting that the diagnosis of malaria may be arrived at

(1) by one's remembering that
     (a) in any case of tropical fever, malaria should be suspected,
     (b) that other fevers in the tropics may resemble malaria ;

(2) on the result of blood examination ;

(3) on the clinical symptoms and signs ;

(4)   on the effect of therapeutic doses of quinine;

(5)   on the previous history of the patient of having visited a malarious place.

From what I have 'said it is evident that " there is no sign or symptom of disease of the human body which malaria cannot simulate." '

THE DIFFICULTIES IN THE TREATMENT OF MALARIAL FEVER *

1. The difficulties in the treatment of malaria and how to combat them.

The types of cases that present difficulties may be grouped as follows :—

(1) Cases in the algid stage of pernicious malaria.

(2) Cases that have marked idiosyncrasy to quinine.

(3) The chronic recurring types of the disease especially benign and quartan malaria.

(4) The possibility of existence of quinine fast parasites(?)

(1) A patient comes under your observation in a collapsed state, temperature is subnormal with cold clammy sweats and no pulse at the wrist and the blood full of malignant tertian parasites. How are we to treat such a case? To give quinine to such a patient by the mouth or even intra-muscularly will do him no good. To my mind the only recourse that must be had in such a case is to give intra-venous injection of quinine and the problem at once arises :— In what dose and in what concentration is one to give intravenous injection of quinine in such a case?

The problem of fall of blood pressure that sometimes follows intravenous injection of quinine especially in concentrated solution at once comes to the mind in the consideration of the treatment of such cases and the most experienced malariologist may hesitate to decide what to

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do. One may give without much hesitation an intravenous injection of a concentrated solution to a patient whose blood pressure is normal and whose heart is powerful but to give such an injection to a patient in the algid stage of pernicious malaria is dangerous and may even lead to fatal results. ' I can recall to my mind at least two cases in which fatal results followed the administration of concentrated solution of quinine in the algid stage of malaria. This brings me to consideration of the changes in blood pressure after intravenous injection of quinine and I cannot do better than refer you to a paper of mine published in the Lancet about two years ago.

R. McCarrison and J. W. Cornwall have shown that all salts of quinine produce a profound fall of blood pressure in sheep after intravenous injection. Many observers have advocated the treatment of malarial fever with intravenous injection of concentrated solution as a perfectly safe method of administering the alkaloid. This method was frequently used during the war in Mesopotamia and other countries where troops were attacked by malaria. So far as I am aware there are no records of the systematic observations on this subject. My own obervations were made in the wards of the Campbell Hospital, Calcutta.

It may be pointed out here that during attacks of malarial fever the blood pressure is generally low, especially in the pernicious type of cases. At the same time, it is in these latter cases that one looks for the most rapid introduction of quinine into the system ; one is therefore tempted to administer the drug intravenously. If there is a profound fall of blood pressure during its administration, then the operation is dangerous and may even prove fatal. A recent writer on malaria has attributed cases of sudden death in such cases to introduction of large doses of saline and considers that quinine should always be given in the concentrated form. I cannot agree with this view.

I carried out certain investigations in order to determine the changes in blood pressure during intravenous injection of quinine, which were described in the Calcutta Medical Journal, February, 1924.

From these observations, the following conclusions can be arrived at :—

(1) Intravenous injection of quinine in concentrated solution (10 grs. in 20 c.c.) is generally followed by a fall in blood pressure and may be followed by disappearance of the pulse for a few seconds.

(2) Intravenous injection of quinine in dilute solution (10 grs. in 200 c.c.) may be followed by a fall in blood pressure, but this fall is neither so sudden nor so great as in the case of concentrated solution. In many cases there is no fall of blood pressure.

(3) The slower the injection is given, the less is the chance of fall of blood pressure taking place.

(4) The diminished blood pressure may persist* for 12 hours or more after the injection.

(5) Intravenous injection of quinine should always be given in very dilute form (1 in 300) and at the rate of 10 c.c. every minute. It should never be lightly undertaken.

(6) Intravenous injection of quinine in concentrated solution may be followed by transient muscular twitchings and quickness of breathing.

(7) Intravenous injection of quinine should be given after making frequent and careful blood pressure observations during the operation.

(8) As in malarial fever especially of the pernicious type, blood pressure is sometimes very low, intravenous injection of quinine should be given very slowly in a dilute form, guarded by administration of pituitrin or adrenalin and application of tight bandages over the extremities.

If the above precautions are taken the dangers of intra-venous injection of quinine will be reduced to a minimum.

From the above considerations, it is clear that the treatment of the algid type of cases of malaria should be intravenous injection of quinine in a dilute solution (1 in 300) and at a rate which should not be more than 10 c.c. every minute. It is also desirable that in such a case the quantity must not be very great as the introduction of a large quantity of fluid into the veins of a patient suffering from the algid stage of malaria may lead to œdema of the lungs and other tissues of the body similar to what occurs during the condition of shock in which it is useless to inject a plain saline solution. But suppose that to the saline we add some colloid which will not pass out of the blood vessels but exert an osmotic pressure of its own, then on the injection of this mixture the blood pressure will be maintained and the necessary circulation of the blood corpuscles kept up for a considerable time. A suitable nontoxic colloid for the purpose is furnished by gum-arabic, so that gum-saline or a gum-glucose-saline may be advantageously administered, in cases of algid type of malaria, together with the quinine. The osmotic pressure of the gum in the fluid for injection should be the same as that of plasma proteins, amounting to 30-40 m.m. of Hg. In any case, the amount of quinine injected should not be more than 10 grains and the operation should be continued with frequent and careful blood pressure observations, guarded by administration of pituitrin or adrenalin. If the case has not gone too far, then the patient may recover. I consider the latter fact as also very important, as I am of opinion that a stage may be reached in such cases when administration of quinine may be useless and I shall mention here the notes of one such case : Patient had malignant tertian infection and was treated as a case of typhoid fever for seven days. On the morning of the 7th day of illness, patient became suddenly restless and quickly passed into an algid stage. I was asked to see the case in the evening. I had

the blood examined which showed malignant tertian infection. His blood pressure was very low and the pulse very quick. I decided to give intravenous injection of quinine in a diluted solution and .10 grains were injected dissolved in 100 c.c., the operation lasting about half an hour. There were no ill effects so far as injection of quinine was considered but the patient died about 4 o'clock in the morning, about 7 hours after the injection. I consider that in this case the capillaries of the brain of the patient were so much clogged with parasites, leucocytes and the debris, that the solution of quinine could not reach the parasites when the intravenous injection was given. It has also to be remembered that Ramsden and Lipkin have found that there are regions in the vascular system that are constantly free from quinine throughout a period of quinine treatment and if the parasites have moved to these regions, intravenous injection of quinine will do little or no good. It is therefore evident that the physician must make up his mind to treat such a case as quickly as possible and not let the patient pass into a hopeless state and beyond any chance of recovery. A case like this recently died in my wards, who was treated with intramuscular injection of quinine. In severely toxic cases may I suggest blood letting which will remove some of the parasite-laden corpuscles?

(2) *The next type of cases that present difficulties in treatment are cases with marked idiosyncrasy towards quinine.*

One serious idiosyncrasy in certain individuals is their liability to attacks of quinine hæmoglobinuria. Such cases have been described by me in the Indian Medical Gazette, Vol. LVI, June, 1921, to which I would refer you.

Another very distressing idiosyncrasy met with in certain individuals is *urticaria*. Some individuals cannot take quinine on this account and prefer to endure

the disease rather than suffer from the intolerable irritation induced by the remedy. I shall not enter here into the mechanism of the production of urticaria under different conditions. Here, perhaps, research should be directed towards the discovery of alkaloids allied to quinine and it is possible that some synthetic derivatives of alkaloids of the cinchona bark may, at some future date, replace quinine in such cases. In some cases aristochin has been found to be less liable to give rise to urticaria than ordinary preparations of quinine. I would further suggest that in all such cases the patient should be on light diet such as milk, and the bowels should be fairly moved and inflammatory conditions of the gastro-intestinal tract corrected. The patient should be given calcium chloride with large doses of alkalies, such as sodii bicarbonas. The subcutaneous injection of adrenalin chloride (10 to 20 m.), repeated if necessary, may be useful. Hiran and others have recommended, in some of these cases which show anaphylactic symptoms with quinine, the following procedure : A defensive dose of $\frac{1}{8}$th grain of quinine sulphate mixed with 8 grains of sodium bicarbonate is given, followed by $1\frac{1}{2}$ grains two hours later. The second day, two hours after the defensive dose, 3 grains of quinine and on the third day, two hours after the defensive dose, 6 grains and so on.

(3) *The next type of cases of malaria which present difficulties in the treatment are the chronic recurring types of the disease especially those of benign tertian and quartan malaria.*

The problem of the occurrence of relapses in malaria has not yet been completely solved. I shall only briefly touch here upon the various views that have been advanced to explain the origin and development of relapses. These are : (1) The parthenogenetic view originally suggested by Grassi and frequently associated with the name of Schaudin. (2) The view of Bignami and Ross which holds that schizogony goes

on throughout the intervals between relapses. According to this theory there is no essential difference in genesis between recrudescence and relapses which occur after long, healthy intervals. By a process of natural selection, schizogony is kept up in the relapsing cases, but the numbers that survive at any given time during periods of apyrexia are too small to produce symptoms or to be found in the peripheral blood. (3) Merozoites may penetrate endothelial cells and adapting themselves to altered nutritive conditions enter upon a resting stage. These endothelial cells are thrown into the blood stream from time to time. Under conditions favourable to nutrition these cells break up, the parasites are set free, and multiply and set up a relapse. (4) Celli and James have advanced the view that relapses depend upon the formation of specialized resistant asexual forms. Biologically two factors have to be taken into account in considering the relapses : (1) According to a general law the propagative phase in the case of malaria, *i.e.*, the gametocyte which provides for the continuance of the species is produced when the environment begins to be unfavourable to the asexual multiplicative phase of the parasites. On the other hand, in a relapse, the environment is more favourable to the multiplicative phase of the parasites. (2) Relapses in a protozoal disease indicate the stages in the progress towards the establishment of complete adaptation between the host and the parasite.

In one word, we may assume that in relapses, a favourable medium is established in the host for the development of the asexual forms of the parasites. Such favourable medium may be the loss of the anti-bodies which resist the development of the asexual stage of the parasites of their pyrogenetic action. This may be brought about by poverty, exposure to cold, debilitating conditions, etc., which may interfere with the defensive mechanism of the host. Perhaps interference with the secretion of the endocrine

glands may also lead to relapses. These are speculations upon which it is not my intention to dilate any further.

Clinically, relapses may be divided into three classes : (1) Relapses at short intervals. (2) Relapses at long intervals. (3) Relapses during vigorous quinine treatment.

The ideal treatment of relapses no doubt should be the complete destruction of the asexual stages of the parasite and in Schaudin's view, complete destruction of their sexual stages also. The means at our disposal for the destruction of the sexual stages by the use of drugs are very limited but there is no doubt that better environments, better climatic conditions and better nutrition to the individual lead slowly to disappearance of these forms of the parasite. In the struggle for existence, the host gets the upper hand and slowly destroys these bodies. Various drugs have also been advocated for their destruction. Arsenical preparations in the form of Salvarsan or Neosalvarsan have been tried, but their action seems to be doubtful. Tartar emetic was advocated by Rogers and others. But as has been shown by myself and others, it is quite useless. I cannot say whether the new antimony compound, Urea Stibamine, would prove effective in the treatment of relapses of malaria.

To my mind, the problem of the treatment of relapses resolves itself into one of suitable method of administration of quinine and since I have conducted some investigations in this direction, I shall refer to this aspect of the treatment in some detail.

Can we ever attain therapia magna sterilans in the treatment of malarial fever by the administration of quinine? Perhaps the answer is in the negative in many cases. It, however, appears to my mind that the failure of quinine to bring about this ideal effect is to some extent due to failure of administering quinine in the most suitable way. If it were possible for us to administer quinine in such sufficient strength that the threshold concentration in the blood will be so great

as to kill all the parasites present, then such an object may be attained. Even if such a threshold were possible to be obtained, one has to consider the observation of Ramsden and Lipkin that in the vascular system there are regions which are kept almost free from quinine throughout the period of quinine treatment. If the parasites are present in such regions then evidently they cannot be killed by quinine unless they come to regions in the blood vascular system to which quinine can gain access.

Theoretically speaking, in a case of malarial infection, intravenous injection of quinine given for a sufficient length of time and in sufficient doses, a few hours before the expected paroxysms, may lead to complete sterilization of the organs.

In the following case of quartan fever, intravenous injection of quinine on the days of paroxysm for several successive days brought about a cure of a relapsing case :— The patient was admitted into my ward on 30-1-19. He gave a history of recurring attacks of fever coming on every fourth day. He contracted the disease in Assam and had been suffering for more than six months. The blood showed quartan parasite. Spleen extended $4\frac{1}{2}$ inches below the costal arch. TREATMENT.—(1) 5 grains of quinine given intravenously on 2-2-19, three hours before the expected paroxysm. Fever recurred on 5-2-19. (2) 10 grains of quinine given intravenously on the expected days of paroxysm, three hours before the expected attack, i.e., on (1) 8-2-19, (2) 11-2-19, (3) 14-2-19, (4) 17-2-19, (5) 20-2-19, (6) 23-2-19, (7) 26-2-19. RESULT.—No recurrence since. Patient remained free from fever for two months in the hospital after the second course of treatment and has been reported free from fever since, i.e., for more than a year and a half. CONCLUSION.—The parasites have been destroyed and patient cured.

It is not, however, always easy to predict the time of occurrence of an expected paroxysm in a case having

treatment with quinine, as the latter frequently brings about a retardation of the paroxysm. I therefore adopted the intravenous injection of quinine on successive days in a series of cases and the results were equally satisfactory. [*Clinical cases are described in the Calcutta Medical Journal, February, 1924.*]

From the above cases the following conclusions may be drawn :—

(1) In recurring benign tertian infections, 10 grains of quinine must be given intravenously for at least seven successive days to bring about sterilization.

(2) In recurring quartan infections, 10 grains of quinine must be given intravenously for at least seven days to bring about sterilization. In one case sterilization was brought about by giving the injections on the expected days of the paroxysm, and in another case by giving the injections for seven successive days.

Such happy results are not always met with and it is not infrequent to find cases in which intravenous injection of quinine fails to bring about complete sterilization and thereby prevent relapses. This is partly due to one's not giving intravenous injections at the correct time. I consider that sporulation in benign tertian or quartan infection begins about two hours before the patient shows definite symptoms of rigor and fever and since most observers are agreed that quinine is most effective against the stage of the growth of the parasites which follows sporulation it is best to give quinine two or three hours before the expected paroxysm so that the amœboid forms may be attacked by the quinine and destroyed. On the other hand, it has been observed that quinine administered intravenously disappears from the peripheral circulation in a very short time. It must, therefore, have gone to the internal organs and if the parasites are concentrated in the internal organs, then the effect of quinine should be concentrated on such parasites and they will be killed.

In most cases, after intravenous administration of quinine for ten days I followed treatment by administration of quinine by the mouth in 5 grain doses, thrice a day, for a fortnight and it appears to me that this line of treatment affords the best chances of cure of relapses.

Before we leave the subject-matter of my lecture, I would refer briefly to quinidine which has recently been advocated by some observers in the treatment of malarial fever in preference to quinine. I cannot do better than refer to a recent discussion that was held on quinidine therapy from which it will be seen how uncertain the drug is in its action. Sometimes there may be sudden collapse with unconsciousness and failure of respiration after a small amount of the drug has been taken. Other unpleasant symptoms are headache, nausea, diarrhœa and vomiting. It exerts a profound effect upon the cardiac musculature and it is not a drug to be used haphazardly and without thoughts. It is a treatment that should be adopted with careful observations in the wards and not in the outpatient departments. Sometimes there may be excessive vomiting that may lead to dangerous collapse. There may be a fall in blood pressure. In essence, it is a powerful depressant and poison to the cardiac muscle. It is, therefore, evident that the exhibition of this drug to patients suffering from malaria with cardiac weakness is likely to be fraught with grave danger.

A drug so uncertain and sometimes so dangerous should not, therefore, on any account be allowed to replace quinine or to be made over to the general practitioner for the treatment of malarial fever.

(4) *Do quinine-fast parasites exist ?* If they do, then it is our despair. But further researches must be conducted to prove that they exist. To my mind, the so-called quinine-fast parasites may be partly due to :

1. Insufficient use of quinine.
2. Improper methods of administration of quinine.

3. Localization of the parasites in regions of the body which may not be approachable to quinine.

4. Want of optimum reaction of the tissue fluids in which quinine acts best against the plasmodium.

From what I have stated, it appears much has yet to be discovered in the treatment of relapses of malarial fever, but the method advocated by me has, in certain cases, given very good results in my hands.

I have just now referred to an optimum reaction of the blood in which quinine acts best against the plasmodium. An ancient practice is to give full doses of alkalies, such as bicarbonate of soda, to reinforce the action of quinine. Perhaps this can be explained on modern biochemical conception as being due to the fact that quinine acts best against the parasites at a certain hydrogen-ion concentration. This method of administration of quinine had recently been advocated by Sinton whose researches may lead to very fruitful results in the future.

I feel it would perhaps have been more correct if I had named the subject matter of my theme to be " *Difficulties in the treatment of malaria and how far we can combat them.*" If, however, you think from what I have stated above that I have succeeded in making some advance in the combating of the difficulties, however small that advance may be, I hope you will pardon me for having given such an ambitious name to the subject matter of my lecture.

THE TREATMENT OF MALARIAL FEVER IN INDIVIDUALS SUSCEPTIBLE TO ATTACKS OF BLACK WATER FEVER BY INTRAVENOUS INJECTION OF AN ANTI-HÆMOLYTIC QUININE SOLUTION

At the meeting of the Medical Section of the Indian Science Congress held in Calcutta last February, I opened a discussion on the treatment of cases of malarial fever, which were susceptible to attacks of black water after administration of ordinary solutions of quinine. I described one such case in which fatal result followed the administration of quinine bihydrochloride intra-muscularly on two successive days in doses of 5 and 9 grains, respectively.

In the discussion that followed at the meeting it appeared that the treatment of cases like the above was one of the most difficult problems in tropical medicine.

It appears to me that the whole problem resolves itself into one of administration of quinine in such a form that should not only have no hæmolytic properties but should be able to exert an inhibitory action in a hæmolytic system. At the time of the above discussion, I did not know whether such a solution could be prepared.

Cases of hæmoglobinuria following administration of quinine in malarial individuals do undoubtedly occur. In some cases, the appearance of the hæmoglobinuria may be

accidental. Leaving these accidental cases and without entering into the discussion of the mechanism of black water fever, hæmoglobinuria in malarial patients after administration of quinine may be due to one of the following causes :—

(1) The quinine radicle or the acid portion of a quinine salt may be responsible for the hæmoglobinuria in susceptible individuals.

(2) Quinine or its salts may, due to some unknown cause, lead to the production of a hæmolysin in the blood of certain malarial individuals.

(3) Quinine may, under certain circumstances, undergo such chemical changes in the blood of a malaria-stricken individual as may give rise to a derivative which may possess hæmolytic properties.

I shall just mention a few facts about the hæmolytic properties of quinine salts, in vitro, from some observations of mine.

(1) Quinine Bihydrochlor.—most hæmolytic of the following quinine salts, quinine bihydrochloride, quinine hydrochloride, quinine bisulphate and quinine sulphate.

(2) Quinine hydrochlor.—less haemolytic than quinine bihydrochlor.

(3) Quinine bisulph.—less hæmolytic than quinine bihydrochlor.

(4) Quinine sulph.—less hæmolytic than quinine bisulph or quinine hydrochlor.

(5) The hæmolytic properties of a quinine salt are diminished by the addition of 10 per cent glucose to the solution of the quinine salt in the normal saline.

(6) Quinine base has very little hæmolytic properties. From the above it will be seen that a non-hæmolytic quinine solution should not contain a quinine salt and it is an advantage to have in it 10 per cent glucose.

I have recently tried to prepare various solutions of quinine base without the aid of any acids, and after a

very extensive series of experiments, I have found that the following solution of alkaloidal quinine possesses very marked anti-hæmolytic properties (it may be stated here that quinine base is very little soluble in water) :—

(1) Quinine base, 5 grains.
(2) Alcohol, 50 minims.
(3) Urethane, 3 grains.
(4) Calcium Chloride, 7·5 grains.
(5) Glucose, 300 grains.
(6) Normal saline, 200 c.c. (0·85 per cent solution of NaCl in distilled water).

The above will give a solution of alkaloidal quinine of the strength of 1 in 600 in normal saline with 10 per cent glucose.*

The following observations will show that the above solution is non-hæmolytic while a solution of quinine bihydrochloride containing the same strength of quinine radicle is hæmolytic :—

(1) 5 c.c. of the solution + 1 c.c. of 5 per cent suspension of erythrocytes = no haemolysis.

(2) 5 c.c. of a solution of quinine bihydrochloride having the same strength of quinine as in the above + 1 c.c. of 5 per cent suspension of erythrocytes = hæmolysis.

The same solution also exerts marked anti-hæmolytic action on a hæmolytic system. Thus it was observed :

(1) ·2 c.c. of the solution + ·8 c.c. saline + a hæmolytic system (1·5 c.c.) = partial H.

(2) ·5 c.c. of do. + ·5 c.c. saline + a hæmolytic system (1·5 c.c.) = slight H.

(3) 1 c.c. of do. + a hæmolytic system (1·5 c.c.) = no hæmolysis.

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* It will be seen that the percentage of alcohol present in the above solution is much less than that in the solution recommended by MacGilchrist (*Indian Journal of Medical Research*, Vol, I, No. 2, October, 1913).

On theoretical grounds this solution, therefore, should exert an inhibitory action on a hæmolysis that may develop in the blood of a patient suffering from malarial fever.

Quinine base given by the mouth is converted into a salt with hydrochloric acid or bile acids before absorption and since the quinine salts possess more or less hæmolytic properties, its oral administration to individuals susceptible to attacks of black-water fever should be objectionable. I consider such individuals should always be treated with the above anti-hæmolytic quinine solution given intravenously.

This solution of quinine base is alkaline in reaction and is very well borne by malarial patients. If its anti-hæmolytic properties are also exhibited *in-vivo*, then I consider that one of the most difficult and vexed problems in malariology will be solved and that many va luable lives may be saved which will otherwise be lost because quinine salts cannot be administered to them on account of their susceptibility to attacks of black-water fever or because such individuals may develop hæmoglobinuria after their administration. I have observed that solutions of salts of quinine lose, to some extent, their hæmolytic properties when they contain 10 per cent glucose and 25 per cent $CaCl_2$, but of all the quinine preparations, the above solution of alkaloidal quinine possesses most marked anti-hæmolytic properties.

It is generally stated that in a case of black-water fever quinine should not be administered if malarial parasites are not found in the blood. But it has to be remembered that the parasites may disappear from the peripheral circulation after administration of a small dose of quinine and be still present in the internal organs. Therefore, the problem whether quinine should be stopped or not cannot be determined by the absence of parasites in the peripheral circulation. If, however, the above anti-hæmolytic quinine solution

is administered, the contra-indications to the administration of quinine are removed even when black-water fever has appeared.

In view of the remarkable anti-hæmolytic properties of the above solution, I invite the workers in the Dooars and other parts of the world where black-water fever is frequently met with, to give it a trial.

As stated before, the above solution is well-borne by malarial patients and I have found that given intravenously it does not lead to such profound fall of systolic blood pressure as is observed in the case of quinine bihydrochlor. I have, sometimes, even found that there is a tendency towards a rise of systolic blood pressure. Frequently, there is a rise of diastolic blood pressure similar to what is observed in the case of quinine bihydrochlor. On the whole, as far as my present observation goes, I have found that circulatory disturbances are less marked in the case of this solution given intravenously than in the case of quinine bihydrochloride dissolved in normal saline.

In order to test whether the hypertonicity of the above solution may lead to any other untoward results, I have found that given intravenously into rabbits, it did not give rise to any such effects. 10 c.c. of the solution ($=\frac{1}{4}$ grain of the quinine base) given intravenously into rabbits weighing 450 to 470 grams did not produce any ill effect. This will correspond nearly to giving 1,200 c.c. of the solution to a man of average weight.

The addition of $CaCl_2$ and glucose was primarily intended partly to help the solution of the alkaloid, partly to inhibit the hæmolytic action of the quinine solution, if there be any, and partly to inhibit the action of any hæmolysin that may develop in black-water fever. They also possess another advantage as they are stimulants to the heart. As far as I am aware, the property of glucose helping the solution of quinine base is a new observation. Urethane

is added also to help the solution of quinine in the same way.

The addition of cholesterin, if it could be kept dissolved in the above solution, would be an advantage in adding to the anti-hæmolytic properties of the above solution. Cholesterin is soluble in alcohol but the amount that would dissolve in the dilute alcohol present in the above solution, is merely a trace.
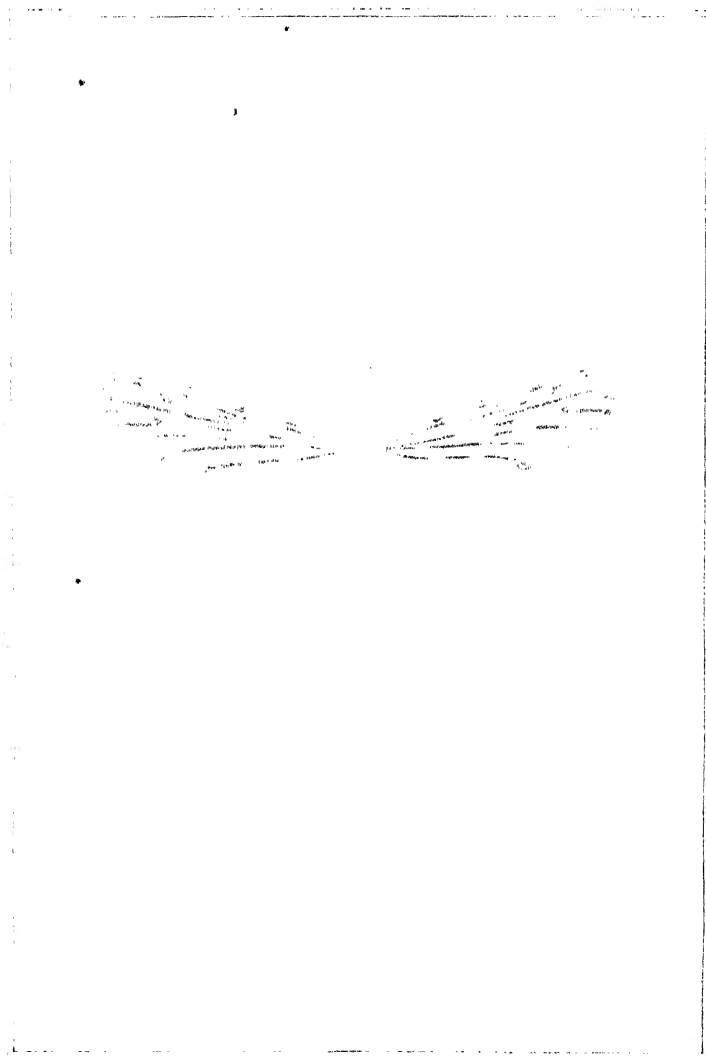
An anopheline allied to Myzomyia listoni

ON AN ANOPHELES ALLIED TO MYZOMYIA LISTONI

This species is small and dark.

Palpi.—With two white bands, the one which includes the tip being much broader than the basal band. The former includes the two terminal segments of the palpi.

Proboscis.—Slightly longer than the palpi, dark brown in its inner half, lighter in colour in its outer half, the tip being lightest in colour.

Antennæ.—With short whitish and blackish hairs.

Head.—Same as in Myzomyia listoni.

Thorax.—Same as in Myzomyia listoni.

Wings.—The costa has five dark-scaled areas separated by small white spots. The first area at the base of the costa corresponds in length to half the corresponding dark area on the costa of the Listoni. The first longitudinal vein has four dark areas, the first being almost equal in length to that of the corresponding area in the costa. The trunk of the second longitudinal vein is white except for a tiny black area at its middle, and another larger dark area near its bifurcation. The anterior branch has three white spots and the posterior two white ones. The third longitudinal vein is white-scaled except near its termination. The fourth longitudinal vein has two white-scaled areas on its trunk. The posterior branch is white. The fifth longitudinal vein has its stem white-scaled except for a tiny spot near its origin. The anterior branch has three white spots and the posterior is almost white-scaled throughout, except for a tiny spot near its base. The

sixth longitudinal vein is white-scaled except for three tiny black areas. The wing fringe is white-scaled opposite all the longitudinal veins including the sixth.

Legs.—Brown, but have white scales at the junction of the femur with the tibia and at the tibio-tarsal and tarsal segments. The white scales are most marked in the anterior leg and less marked in the middle and posterior legs.

Abdomen.—Same as Listoni.

Locality.—Found in a tank in the Campbell Hospital, Calcutta, among large number of fuliginosus. The tank has grassy sides.

This Anopheles differs from true Listoni in the following respects :—

1. Different palpal markings.
2. Different proboscis markings.
3. Different wing markings.
4. Different leg markings.

ON SOME NEW ANOPHELINES OF CALCUTTA AND ON THE · SEASONAL PREVALENCE AND VARIATIONS OF ANOPHELES FULIGINOSUS OF CALCUTTA

In their reports to the Malaria Committee (1902), Stephens and Christophers describe the prevalence of the following species of Anopheles in Bengal :—

A. rossi, A. fuliginosus, A. sinensis, sub-sp. nigerimmus, A. lindesayi, A. metababs and A. christopheri. Of these, they found rossi, fuliginosus and nigerimmus in Calcutta and certain of its outlying portions. Subsequently, Alcock collected some listoni in Calcutta and Adie in a private communication tells me that he found some listoni in the tank of the Indian Museum.

In their Monograph on the Anophelines of India (Second Edition), Liston and James mention the presence of the following additional species in Calcutta : Myzorrhynchus jamesi and Myzorrhynchus barbirostris.

My work on the Anophelines of Calcutta extends over a year and during this period I have discovered the following additional species in Calcutta :—

The first of these is Myzomyia ludlowi. It is allied to M. rossi but has speckled legs. Recent investigations of Christophers have proved this to be the carrier of malaria in the Andamans.

The second new species is M. culicifacies. It is allied to M. listoni, but differs from it in some important points such as, fine dark areas on the costa, black-scaled third

longitudinal vein, presence of only three white patches on the costa including the one at the apex, etc. It is a very efficient malaria-carrier in nature.

The third new anopheles is the one, a specimen of which was exhibited by me in the April meeting of the Asiatic Society of Bengal last year and subsequently described in the July number of the *Indian Medical Gazette*. This belongs to a new species which has been designated as M. brahmacharii by Christophers. Its great peculiarity is that its proboscis is white-scaled in its outer half. In their Monograph, Liston and James point out that, so far as they are aware, Nyssomyzomyia punctulata is the only anopheles which is white-scaled in its outer half. This new myzomyia is, therefore, the second species of anopheles in India which has also got the same characteristic.

All the above myzomyias were found in the tank of the Campbell Hospital, ludlowi being found in from November to February, culicifacies in February and brahmacharii in February and March.

I have also found listoni in the same tank in which there is no running water, just as Alcock and Adei found them in the tank of the Indian Museum. Listoni were found from October to March.

The largest number of stephensi were found in a masonry reservoir containing water for washing cooking utensils.

Contrary to the observations of Stephens and Christophers, I found A. fuliginosus to be the most common anopheles in Calcutta.

Out of nearly 12,000 larvæ caught from July to January, about a ninth developed into the adult stage, the remaining having died. This probably gives us an idea of the enormous number of larvæ that do not pass to the adult stage. It would be most interesting to observe the influence of seasonal variations on the natural destruction of anopheles in the larval stage.

Seasonal Variations of Fuliginosus of Calcutta

The characteristics of fuliginosus of Calcutta :

(1) The costa has six long black-scaled areas separated by white spots.

(2) There is a frequent tendency to the occurrence of long white bands in the femur and tibia and sometimes in the first tarsal segment in the ventral and lateral aspects of the legs. These bands are parallel to the long axis of the legs.

(3) Frequently, there are no white bands or scabs at the junction of the 4th and 5th tarsal segments in the forelegs. Similarly in the mid-legs there are generally no white bands or scabs at the junction of the 3rd and 4th tarsal segments as well as of the 4th and 5th tarsal segments.

(4) The third longitudinal vein is generally white-scaled in the middle of its course, but sometimes, without any other seasonal variations, it may be black-scaled, especially in winter.

(5) The tip of the fifth tarsal segment in the hind leg sometimes contains a minute black spot.

(6) The peculiar seasonal markings of the tarsal segments of the hind legs, which I shall describe presently.

The typical fuliginosus of Calcutta has three tarsal segments perfectly white in the hind legs. As winter approaches, faint dark spots appear in the proximal ends of the third tarsal segment. These spots increase till half and sometimes almost the whole of the segment becomes black-scaled.

The tip of the fifth tarsal segment is more frequently found to have a minute black spot during winter. In some cases, almost the whole of the fifth tarsal segment in the hind leg is found black during winter. In this season, the third longitudinal vein is more black-scaled in the middle of its course than white.

Contrary to what is found in Adei, the palpi of fuliginosus of Calcutta are always the same as in the type, the palpal bands being always three. The seasonal variations are not so constant as in Adei. While it is more frequent that in winter the third longitudinal vein is more frequently black and the third tarsal segment in the hind leg also tends to be black, we find that this is not invariably the case, nor is the amount of black colour constant and sometimes this may be completely absent.

Lastly the junction of the third and fourth tarsal segments in the mid-leg is frequently found to be black throughout the year.

The A. fuliginosus of Calcutta differs from Adei in the following points :—

(1) The palpal bands are always three and never four.

(2) The junction of the third and fourth tarsal segments in the mid-leg is more frequently black-scaled and only occasionally white-scaled.

(3) *The tip of the fifth tarsal segment in the hind leg has sometimes a minute black spot, especially in winter and sometimes the whole of the segment tends to be black.*

(4) The seasonal variations are not so constant as in Adei.

Seasonal Prevalence of Fuliginosus of Calcutta

The method by which I have estimated the anophelines in a locality depends upon careful daily larval counting from the breeding places. I already described this method in the meeting of this section of the Asiatic Society last April, and subsequently in the meeting of the Central Malaria Committee, held in Bombay last November.

Assuming that the number of adults are proportional to the number of larvæ caught, I have drawn the accompanying curve from the monthly larval counting of A. fuliginosus.
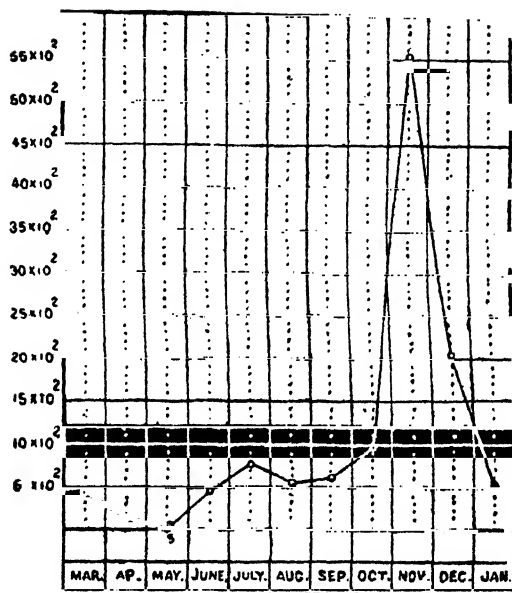
Curve of monthly larval counting of Anopheles fuliginosus
(seasonal prevalence)

It will be seen from this curve that the number of A. fuliginosus is lowest about May and highest towards November. There is also a rise in their number in July.

It must, however, be mentioned that the sides of the tank, from which the larvæ were collected, were cleared out in May and December, and the diminution in the number of larvæ caught may have been partly due to clearing out of the weeds. That this is not the only cause of their diminution is borne out by the fact that the number began to diminish before the cleaning of the sides of the tank was started.

The highest rise in the anopheline curve in Calcutta seems to correspond to the greatest prevalence of malaria in Bengal, *i.e.*, in November.

The numerical determination of anophelines in any locality is a very important matter for malariologists to study, as by this we can forecast the occurrence of intense or epidemic malaria. As there are no accurate methods for their determination, it would be very interesting if observers would test the accuracy of the method described by me—a fact that can only be settled by careful and laborious observations for several successive years and this is what I myself also propose to do.

A PRELIMINARY REPORT ON OBSER-VATIONS OF THE HABITS OF ANOPHELES

Our observations began from the month of July (1901) and the present article is only a preliminary report on our observations.

Breeding places of anopheles during the rainy season

House-drains and those along the sides of streets or lanes rarely shew any larvæ of anopheles though culex larvæ abound in them. Water collected in places having weeds or grass growing over it seems to be a special attraction for the anopheles to breed in. Places containing water a few inches deep having mud at the bottom and protected from the wind, the rain and the sun by grass or water-plants, are often selected for breeding sites. Small watercourses in parts where the water is stagnant, shaded and covered by decayed grass and bits of straw or rotten banana leaves or trees floating on their surface, seem to be another favourite site for breeding. In a watercourse we found while examining the water one morning that a portion of it, where the rays of the sun were directly falling and not shaded, showed far fewer larvæ than in those portions a few feet apart, which were more shaded. On another occasion the removal of the waterplants and the grass from the sides of a small *kutcha* surface-water drain containing a number of anopheles larvæ was followed by their complete disappearance for about a month and a half, and at the time of writing they have again been found at places where the grass has begun

to grow. The selection of the sites for breeding was shown by no larvæ being found in iron cisterns filled with water and kept near the breeding places, nor in earthenware vessels kept in the rooms infested by the adults. Even in sites containing the larvæ we were struck by their unequal distribution. In a surface-water drain the largest number were found in the narrowest, the most shaded and the most shallow parts.

In tanks, too, the selective power of the anopheles in choosing its breeding sites is markedly shown. We examined two tanks under almost similar conditions and a few yards apart from each other, and found that anopheles larvæ abounded in the one which was partially covered by weeds, while in the other, which was free of all water plants, none was found. Tanks covered by such water-plants constitute a favourite site for breeding.

Running water does not seem to be a favourite breeding site, though many larvæ are found at the sides of watercourses where the water is flowing in a slow stream. In such a place the water at the sides, though not actually running, is being slowly renewed.

Merely shaded places containing water do not necessarily constitute a breeding place of the anopheles. Many housedrains perfectly shaded from the sun never show any larvæ. Even in watercourses the parts shaded by big trees growing from the sides but not covered by water-plants show far fewer than those where the water-plants were present. A *pucca* house-drain, however clean or dirty it may be, has not been found by us to be infested by anopheles larvæ though culex larvæ abound in such places.

Anopheles larvæ have been found in places containing fish, though in tanks containing a large number of fish they are absent, unless there is protection afforded to them by the presence of water-plants. Culex larvæ have often been found in the breeding places of anopheles, but generally the

former are found in much smaller numbers in places which are the favourite sites for the latter. Besides, in a very large number of places containing culex larvæ, no anopheles has been found. It is not merely a question of struggle for existence between the two and survival of the fittest, but an actual selection of the habitat that determines the presence of anopheles in a sample of water. Taking, for instance, an earthenware vessel containing water and kept in a room infested by both anopheles and culex, we have found that in a short time it becomes full of culex larvæ while no anopheles larvæ have as yet been found. We have seen the anopheles sitting on the walls of such vessels probably for the purpose of drinking water, but we have seen them depositing their eggs. Similarly, again, house-drains or drains along the streets containing excess of sewage contamination have been found full of culex, but not of anopheles larvæ. Generally in a place where anopheles larvæ are found in large numbers culex are few, though on one occasion we found both anopheles and culex larvæ in large numbers in the same place.

Anopheles does not necessarily breed in places where the larvæ can be made artificially to live and grow. We took, for instance, the water of a drain containing only culex larvæ with the mud at its bottom, and after all the culex had developed into adults we introduced an impregnated anopheles female into a bottle containing the water. Eggs were deposited and the larvæ that were developed out of them lived for more than a fortnight, though none grew up to the adult stage.

To test whether the anopheles made any selection of water for depositing its eggs, we made the following experiments :—

1. Impregnated anopheles females were introduced into a wide mouth bottle containing 3 gallipots, having wet mud, water, and water covered by green grass

respectively. Eggs were deposited in the wet mud and the water covered by green grass but not in the pure water.

II. Impregnated anopheles females were introduced into a wide-mouth bottle containing 2 gallipots having water and water covered by green grass respectively. Eggs were only deposited in the water covered by green grass.

III. Impregnated anopheles females were introduced into a wide-mouth bottle having water and wet mud respectively. Eggs were deposited only on the wet mud.

These experiments have not been exhaustively made, but so far they distinctly go to prove that the anopheles has a great preference for depositing its eggs in water containing mud or green grass.

Anopheles eggs.—The eggs are deposited at angles with each other forming several equilateral triangles 'joined to each other. Sometimes they do not form any triangles, and sometimes they lie parallel to each other. On a dewy surface, as inside a bottle inverted over another containing water, they are deposited separately from each other.

The method of deposit is quite different from the way in which the culex deposits its eggs, which consists of parallel rows joined to each other and giving rise to a somewhat compact mass slightly concave in the middle. Typical egg boats are, therefore, formed by the culex and not by the anopheles eggs. The eggs are generally deposited in one sitting, though on one occasion we found the deposit of eggs was completed in two sittings with an interval of two hours.

Anophelines may be artificially made to deposit its eggs on any kind of water. Tap water, water from various drains and distilled water were tried, and in all of them the eggs

were deposited and hatched.   Inside bottles we have seen them sometimes depositing their eggs within two hours and at other times after eight to twelve hours. They are deposited at night; sometimes towards evening, and sometimes towards morning. On some occasions the anopheles refused to deposit their eggs without any obvious reason, and we often found that those that did not deposit their eggs on one night refused to do so on subsequent nights. Eggs were never deposited in the day. We have not as yet found them depositing their eggs in the day even when kept in the dark. In perfectly dry tubes we never saw them deposit their eggs as observed by Major Ross, but on a hard dewy surface they may be laid as we found inside a bottle inverted over another containing water.

The eggs cannot bear desiccation for any length of time. Though kept alive in contact with moisture, yet when the water of a bottle containing them is shaken so that they stick to the sides and dry up, they die within a short time. Many eggs are thus likely to be destroyed in nature by strong wind. The eggs are hatched by the separation of a cap. As soon as the cap separates the larva shoots out of the shell unlike the culex which may come out of the shell slowly and may remain coiled inside it for some time after the separation of the lid. The separation of the cap of the egg shells may be facilitated by teasing them gently with a needle, or simply by putting them on a slide with a drop of water at the approximate time of hatching. The *eggs may even be hatched in pure kerosene oil*, if they are put in it at this time. We have made them hatch in a solution of Canada Balsam, and have succeeded in this way in making specimens of larvæ partly inside and partly outside the egg shells.

Anopheles larvæ.—They are generally hatched at temperatures of 84º F to 86º F, within 24 to 30 hours. The

process of hatching of the eggs is completed in five or six hours after it has started.

The larvæ of anopheles are fond of sticking to decayed grass or leaves or bits of straw floating on the surface of water. They have a great attraction for rotten plantain trees. The larvæ of the same, species may differ in colour according to the kind of food they live upon. In bottles containing mud at the bottom they can be seen sinking down to feed themselves. While feeding they lie horizontal or perpendicular to the surface of the mud, to which some of them may remain sticking even when the water is moderately disturbed. Sometimes they penetrate a slight distance into the mud to seek nutrition.

We have never seen the habit of cannibalism among the larvæ, though occasionally the dead larvæ were seen being seized by the living ones. When dead they are seen floating on the surface like a scum or they may sink to the bottom.

The larvæ live in different kinds of water for different lengths of time.

(1) Larvæ developed in simple tap water, kept unchanged, lived for three days in it.

(2) Larvæ, about a week old, were put into a bottle of tap water containing a small quantity of mud—some lived for ten days.

(3) Larvæ developed in tap water containing plantain juice—some lived for about ten days.

(4) Larvæ developed inside a bottle containing water from a drain with the mud in it—many grew fast for some time and some lived from 15 days to three weeks.

(5) Larvæ developed in tap water containing mud from the streets—a few (very much dwarfed) lived for three weeks.

It is extremely difficult to make the anopheles pass through their whole larval stage in water contained in *gumlas* or bottles, if the water is kept unchanged. Various kinds of water, such as, water containing plantain juice, water of places where the larvæ are found with or without the water-plants found in them, and with or without the mud at the bottom, have all been tried without being renewed. In none of them did the anopheles pass through the entire larval stage. This markedly contrasts with the habits of the culex, the duration of the larval stage of which can be easily studied. On one occasion some of the larvæ, caught and kept in the water where they were found, did not complete their larval stage even after three weeks, after which they died, due, probably, to inanition.

Anopheles larvæ have been kept alive in bottles containing mud and water not more than one-sixteenth inch deep showing that they can live in very shallow water.

In all the places where we found the anopheles we also found culex larvæ, though generally where anopheles are found in abundance culex are not plentiful. Rarely anopheles larvæ have been found in places where culex formed the great majority of the larvæ.

Anopheles larvæ do not bear desiccation for any length of time. Larvæ, six days old, were put on a dry slide at a temperature of 92°F. in a breezy place and found dead in 15 to 18 minutes.

Effect of heat upon anopheles larvæ :—

(1) Larvæ—a day old—were not dead in 3 hours when the temperature was raised up to 100°F.

(2) Larvæ—two days old—were kept in water at a temperature of 110°F; most of them died, but a few were alive even up to 20 minutes.

(3) Larvæ—a day old—were kept in water at a temperature of 115°F; all died in 6 minutes.

(4) Larvæ—a day old—were kept in water at a temperature of 115°—117°F ; all died in 2 to 3 minutes.

(5) Larvæ—2 days old—were kept in water at a temperature of 117°F ; all died in ½ to 1 minute.

Effect of kerosene oil and solution of salt upon anopheles larvæ :—

(1) Larvæ—a day old—live from ½ to 2 minutes in pure kerosene oil.

(2) Larvæ—a day old—were kept in water poured over kerosene oil (kerosene oil—2″ and water—2½″ deep) ; death took place in 15 to 20 minutes.

(3) Larvæ—12 hours old—were kept in water 3″ deep, over which kerosene oil was gently poured (1½″ deep) ; many died, but some lived even up to 3¼ hours.

(4) Larvæ—three or four days old—were kept in a saturated solution of salt ; all died in 15 to 20 minutes.

Anopheles pupœ.—The pupa stage lasts from 24 to 48 hours.

Anopheles adults.—All the varieties that have been examined by us do sing. It seems that the song of the males is more high-pitched than that of the females. The males have been kept alive inside bottles containing water with plantain juice for a week, while the females under such circumstances died in one to two days ; on the other hand, in perfectly dry tubes the females live longer than the males, which sometimes die some hours after they are caught. The females soon after birth do not have much attraction for human blood. This was well exemplified in one case in which a large number of new-born anopheles were introduced inside a mosquito curtain with a patient suffering from intermittent fever sleeping under it. It was found next morning that all of them were sticking to that part of the curtain which was accidentally wetted by the rains. The same, we may remark here, holds good in the case of some species of culex too. Anophelines do not seem to fly to long

distances from the places of their birth. They are caught in largest number sitting on the folds of *black* blankets and a much smaller number is found sitting on white walls. Generally in test tubes the males can be seen apparently sleeping at night while the females fly about.

N.B.—The observations in this paper on the habits of anophelines, especially their breeding places, require considerable modification in the light of subsequent experience of the authors as well as of other workers. The only justification for publishing the paper in its original form is just to have a historical record of what the authors found in the early days.—Editor.

SOME OBSERVATIONS ON THE HÆMO-LYSIS OF BLOOD BY HYPOSMOTIC AND HYPEROSMOTIC SOLUTIONS OF SODIUM CHLORIDE

In the Lancet, April 2, 1904, Sir A. E. Wright and Kilner, in describing a new method of testing the blood and the urine, state that when complete hæmolysis takes place a dark coloration is observed in a mixture of one volume of suspension of erythrocytes with one volume of a progressive dilution of a deci-normal sodium chloride solution in a capillary tube. Later on, Wright and Ross[1] point out that instead of making a preparation of the suspension of the red corpuscles, all that is required is to take a measured volume of the blood and to mix with it two volumes of the progressive dilution of the deci-normal sodium chloride solution, and then to observe when the dark coloration takes place.

It will be seen from the above, that it is assumed firstly, that it is possible to bring about complete hæmolysis by mixing one volume of blood with two volumes of a sufficiently dilute solution of sodium chloride, and secondly, that the point of complete hæmolysis can be determined by letting light fall obliquely upon capillary tubes containing the mixture, it being supposed to be completed when the brightness of the solution disappears and a dark colour appears. In this way, Wright and Ross conclude that the

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[1] *The Lancet*, October 21, 1908.

average European blood hæmolyses completely with two parts of $N/35$ sodium chloride solution.

I am unable to agree with the observations of Wright, Kilner and Ross, that *complete* hæmolysis can be brought about in the above way. By treating normal blood with two volumes of $N/100$ sodium chloride solution as well as with two volumes of distilled water, I have succeeded in demonstrating that in none of these is *complete* haemolysis obtained.

I consider that the most accurate conception of complete hæmolysis is that the blood, supposed to be completely hæmolysed, should be perfectly transparent, or if it is not perfectly transparent, it should give, on centrifugalisation, a sediment which, when thoroughly washed with an inactive fluid, should not be red. Further, it should not show the presence of hæmoglobin-containing erythrocytes, which can be stained with proper stains. By an inactive fluid is meant a fluid which has no action on the erythrocytes, and cannot, therefore,. dissolve the hæmoglobin contained in them, but can dissolve any free hæmoglobin.

To determine whether the sediment is *red* or not, the supernatant fluid is to be pipetted off and the sediment treated with a solution of sodium chloride which cannot cause any more hæmolysis in the blood under consideration. The mixture is then centrifugalised again and the sediment separated and treated in the same way as before, and if after a sufficient number of washings, it is found that the supernatant fluid at the top is colourless and the sediment is *red*, then it is evident that complete hæmolysis has not taken place. The sediment may further be tested for the presence of hæmoglobin-containing corpuscles, and stained with a proper stain to show the presence of stained erythrocytes. If, on the other hand, the sediment is colourless, then evidently complete hæmolysis has taken place.

Under ordinary circumstances, an $N/10$ sodium chloride solution will serve the purpose of the inactive fluid mentioned

above. I shall, however, see, later on, that this solution cannot always be used for the above purpose, as, for instance, when the blood has been previously treated with a saturated sodium chloride solution.

I began our investigations by testing different specimens of blood from the healthy students of the Gampbell Medical School, Calcutta. The blood of a large number of students was examined in the above way, the diluting fluid being either distilled water or $N/100$ sodium chloride solution, generally the latter. In none of these cases did I observe complete hæmolysis conforming to the definition given above. In other words, I always obtained a *red* sediment after the blood was treated in the above way. At the same time the dark coloration described by Wright and others was generally obtained with two volumes of $N/40$ to $N/50$ sodium chloride solution.

That the red sediment obtained in the above experiments contains undissolved erythrocytes can be shown in the following way :—

   (1)   The sediment though insoluble in $N/10$ sodium chloride solution is dissolved after being repeatedly washed with $N/100$ sodium chloride solution or distilled water as the case may be.

   (2)   The sediment shows the presence of hæmoglobin-containing erythrocytes under the microscope.

   (3)   The sediment, when stained with a proper stain, shows the presence of stained erythrocytes.

In some of my cases I put the mixture of blood with distilled water as well as the mixture with $N/100$ sodium chloride solution, for nearly twelve hours in corked tubes, and it was found that complete hæmolysis had not taken place even after this period, the temperature of the room being 29°C. during the day.

The question now arises as to how many parts of distilled water or $N/100$ sodium chloride solution can completely

hæmolyse one part of human blood. I have made dilutions of blood several times with one, two, and up to nine parts of distilled water, as well as $N/100$ sodium chloride solution, and have obtained the *red* sediment in all of them after repeated washing of the sediment with $N/10$ sodium chloride solution. The sediment also showed the presence of hæmoglobin-containing erythrocytes, which took easily eosin stain. In one case I diluted a specimen of blood with 40 parts of distilled water, and kept the mixture for twelve hours in a small tube, and could detect the presence of hæmoglobin-containing erythrocytes, easily taking the eosin stain.

The corpuscles containing hæmoglobin, and which are found in the red sediment described above will, in future, be called *sediment corpuscles*.

The sediment corpuscles can be fixed in pure methyl alcohol or absolute alcohol, and stained with a dilute solution of eosin in water, by immersing the slides from twelve to fourteen hours in the solution, or may be stained by mixing the sediment with a dilute solution of eosin in $N/10$ sodium chloride solution.

I append here a plate showing the sediment corpuscles after being fixed and stained in the above manner, they having been obtained by mixing human blood with 2 vols. of $N/100$ sodium chloride solution, and the mixture left undisturbed for one hour at the temperature of the room (29°C.).

A similar phenomenon is seen when blood is treated with nine volumes of $N/100$ sodium chloride solution, except that the sediment corpuscles are much fewer in number and the destructive changes noticed in them are more marked.

The laking of blood by hyposmotic sodium chloride solutions has been supposed to be due partly to osmosis and partly to the specific sensibility of the cortical layer of the erythrocytes or the membranes holding the hæmoglobin within the corpusoles. I consider that at least a third factor

is present upon which the above phenomenon is to some extent dependent. If we examine the sediment corpuscles, it is easily seen that a large number of them have undergone marked changes in the shape, size•and in the amount of contained hæmoglobin. Some of them are resistant in the sense that they have not at all discharged their hæmoglobin. But there are others which show marked diminution in the amount of hæmoglobin. Some show marked changes in the distribution of their contained hæmoglobin, as compared with the normal (see Plate). Evidently in these the cortical layer of the envelope has been ruptured, leading to partial escape of the hæmoglobin. What is it, therefore, that prevents the remaining portion of the hæmoglobin from being completely discharged ? The most probable assumption is that the process is to some extent allied to mass action that takes place in chemical reactions. In other words, there probably exists a union allied to chemical combination between the hæmoglobin of the erythrocytes and other portions of their structure, perhaps including the salts. This combination is probably broken up when water enters their structure, e.g., when they are treated with hyposmotic sodium chloride solutions, but the amount of decomposition will depend upon the relative masses of the interacting compounds.

Resistance of the Erythrocytes to Hæmolysis under Abnormal Conditions

If one volume of normal blood is mixed with two volumes of $N/20$ sodium chloride solution, slight hæmolysis is not infrequently observed, while with $N/30$ it is often distinct or sometimes even marked. In certain forms of anæmia, $N/20$ causes no hæmolysis, while $N/30$ causes very slight or no hæmolysis. In other words, in some forms of anæmia, the blood resists hæmolysis more than normal blood.

Captain McCay by estimating the hæmosozoic value of serum in certain forms of anæmia, also arrives at the same conclusion. He thinks that this might be due to the presence of something of the nature of an antihæmolysin.[1]

Clinical Data of a Case of Anæmia having High Resisting Power to Hæmolysis

Patient, æt. 25, was admitted into my ward on September 16, 1908. Condition on admission—anæmic, slight œdema of the extremities, no albumen in the urine, stools contain ova of ankylostoma. On September 22, he had 2,700,000 red cells and 20 per cent hæmoglobin. One volume of blood plus two volumes of $N/20$ sodium chloride produced very slight hæmolysis. The patient was treated with thymol since admission, but got worse since coming into hospital. He became markedly œdematous, more anæmic, and his condition was considerd hopeless. On November 10, his erythrocytes showed more resistance to hæmolysis, two volumes of $N/30$ sodium chloride plus one volume of blood not showing the slightest amount of hæmolysis. To determine whether this resisting power was due to anything present in the serum, I washed the erythrocytes several times with a deci-normal sodium chloride solution, till the supernatant fluid obtained on centrifugalisation was found to be perfectly free from the slightest trace of albumen. One volume of the suspension of the erythrocytes was treated with two volumes of $N/30$ sodium chloride solution, the resulting mixture did not show any hæmolysis at all. Salinity was 0·585 per cent, and alkalinity, estimated in the way pointed out by Moore and Wilson,[2] was 0·095 H_2SO_4. Red cells —1,770,000; hæmoglobin—13 per cent. It will thus be seen that the resistance to hæmolysis was not

[1] McCay, *Bio-Chemical Journal*, Vol. III, 1908, p. 97.
[2] Moore and Wilson, *Bio-Chemical Journal*, Vol. I, 1906, p. 297.

due to anything present in the plasma, as the same resistance was observed when the serum was replaced by a deci-normal sodium chloride solution.   There was a marked diminution in the alkalinity of the blood, which cannot, however, account for the resistance of the erythrocytes to hæmolysis.

Behaviour of the Erythrocytes of Man and the Rabbit towards Saturated Solution of Sodium Chloride

When one volume of human blood is mixed with fifteen volumes of a saturated solution of sodium chloride in distilled water the mixture at once becomes turbid.   This turbidity is quickly followed by a marked solution of the erythrocytes, and the mixture at the same time becomes clear to a great extent.   With rabbit's blood no such clearing up of the mixture takes place in a short time, and the fluid remains turbid for a longer time.   If the rabbit's blood, after it has been treated in the above way, be centrifugalised within ten minutes, it is found that the supernatant fluid is faintly red, showing that only a slight hæmolysis has taken place by this time, contrary to what is found in the case of human blood in which the supernatant fluid is found to be markedly red.   If, however, the sediment of the rabbit's blood, obtained above, is mixed with the supernatant fluid at its top, it shows the remarkable property of dissolving to some extent, as if some hæmoglobin was squeezed out of the erythrocytes during the process of centrifugalisation. The same sediment, when treated with $N/10$ sodium chloride solution, dissolves to a much greater extent.   It is thus evident that the undissolved erythrocytes are markedly altered in their constitution after the treatment of the blood with saturated sodium chloride solution.   Examined under the microscope they are found to be much contracted, but most of them retain their globular shape and do not look crenated or wrinkled.   The most probable explanation

of this hæmolysis appears to me to be a marked change in the outer walls of the erythrocytes brought about by the saturated sodium chloride solution ; probably a sort of combination takes place between sodium chloride and the outer layer of the erythrocytes which finally leads to its destruction. When the blood is mixed with the saturated sodium chloride solution, no doubt water comes out of the erythrocytes by the process of osmosis and they accordingly contract ; when the sediment from the above is treated with $N/10$ sodium chloride solution, water re-enters their structure, and, as a result of this, they try to expand and regain their original size. But either they burst before or as soon as they recover their original size, or it may be that the water dissolves the compound of sodium chloride on the outer wall of the erythrocytes and consequently a marked hæmolysis results. The initial turbidity, mentioned above, is probably due to the production of the compound, which is probably very easily decomposed. It is evident that osmosis alone cannot explain the hæmolysis of blood by saturated sodium chloride solution. The remarkable pheno-menon of hæmoglobin coming out of the corpuscles during centrifugalisation is probably explained by assuming that the damaged walls of the erythrocytes allow hæmoglobin to pass out of them by a process allied to filtration under very high pressure. As soon as the unstable compound of sodium chloride with the outer layer of the erythrocytes is decomposed, the latter behave like small spheres of sponges containing dissolved colouring matter. .
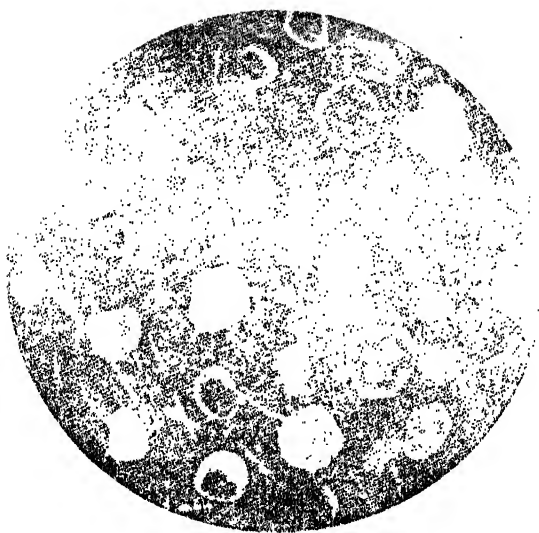
Resistant corpuscles from a mixture of one part of blood and two
parts of N/100 NaCl solution.

ON THE RELATIVE HÆMOGLOBIN VALUE OF THE RESISTANT ERYTHRO-CYTES DURING THE HÆMOLYSIS OF BLOOD WITH HYPOSMOTIC SODIUM CHLORIDE SOLUTION, AND ON THE PERMEABILITY OF THE ERYTHROCYTES TO WATER AS A FACTOR IN THE PRODUCTION OF HÆMOLYSIS

In a previous paper [1] I have pointed out that the dark coloration described by Wright, and obtained by mixing one part of blood with two parts of a progressive dilution of saline does not represent the point of complete hæmolysis. This point is obtained in the observations of McCay [2] and my own observations by mixing one part of blood with two parts of N/40 to N/50 saline solution. It may, for the sake of convenience, be called *Wright's hæmolytic point.*

This point probably represents the stage at which a large number of the erythrocytes undergo hæmolysis as the result of osmosis and rupture. The corpuscles that do not hæmolyse at Wright's hæmolytic point will be termed in this paper the *resistant corpuscles.*

By quantitatively estimating the amount of dissolved hæmoglobin in 20 c. mm. of the supernatant fluid obtained

[1] *Bio-Chemical Journal,* Vol. IV, 1909, p. 59,

[2] *Ibid.,* Vol. III, 1908, p. 97.

after centrifugalisation of a mixture of blood and two volumes of N/x saline solution, where x is any number from 20 upwards, I have made out the *curve of hæmolysis with hyposmotic saline solutions.*

From the curve below it will be seen that the very beginning of hæmolysis starts with $N/20$ saline solution. Then the degree of hæmolysis suddenly increases from $N/20$ to $N/30$ saline. From $N/30$ to $N/45$ or $N/50$ it is somewhat gradual, while from $N/50$ upwards it increases very slightly with the higher dilutions.

The fact that some of the erythrocytes hæmolyse with higher dilutions of saline than others leads to the conclusion that either they are less permeable to water or they can bear the tension of distension from osmosis better than others, and therefore do not rupture so readily. I have however, already pointed out that osmosis and rupture alone cannot explain the whole phenomenon of hæmolysis with hyposmotic saline solutions, and that one has to take the question of mass action into consideration in explaining it. The presence of erythrocytes containing partially discharged hæmoglobin among the sediment corpuscles goes against the theory of rupture.

The relation of the amount of hæmoglobin in the resistant corpuscles to the total amount in the sample of blood under examination appears to me, from observations in health and disease, to have an important bearing, and I would suggest that this be called the *relative hæmoglobin-value of the resistant erythrocytes.* It may be expressed as the quotient obtained by dividing the amount of hæmoglobin in the resistant corpuscles by that of the total blood.

The method by which I estimated the amount of hæmoglobin in the resistant corpuscles is described as follows :—In all cases the blood was hæmolysed with two parts of $N/50$ saline solution, with which in the case of healthy individuals Wright's hæmolytic point is with certainty
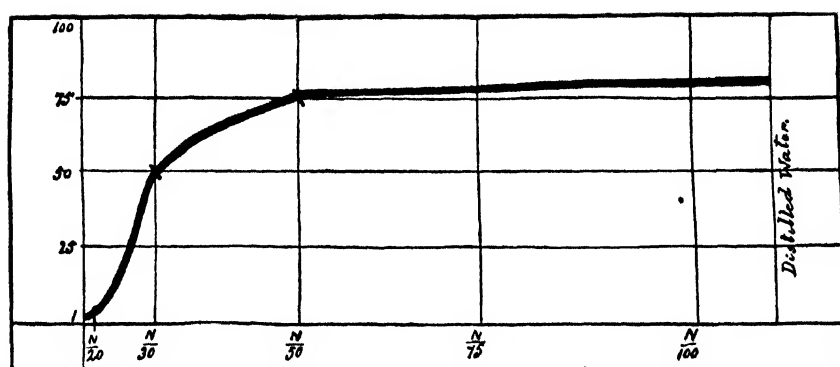
Curve of hæmolysis with hyposmotic saline solution

obtained. After thoroughly mixing 5 c. mm. of the blood with 10 c. mm. of $N/50$ saline the mixture is centrifugalised as thoroughly as possible, and then the sediment is washed several times with $N/10$ saline till the supernatant fluid is perfectly colourless. The sediment is now dissolved in a small quantity of distilled water with the addition of a drop or two of chloroform, and then the amount of hæmoglobin is estimated by Haldane's hæmoglobinometer. In those cases in which the amount of hæmoglobin in the resistant corpuscles is less than 10 per cent, 10 or 20 c. mm. of blood are taken and then treated with 20 or 40 c. mm. of $N/50$ saline respectively, and the amount of hæmoglobin in the resistant corpuscles is then estimated. This number divided by two or four, as the case may be, gives the amount of hæmoglobin in the resistant corpuscles of 5 c. mm. of blood.

The accompanying table gives the relative hæmoglobin-value of the resistant corpuscles in the blood of 'some of my students as well as in some cases of anæmia in my wards :—

TABLE I—HEALTH

Hæmoglobin in 5 c. mm. of blood		Hæmoglobin in the Resistant Corpuscles in 5 c. mm. of blood		Relative Hæmo-globin-value of the Resistant Corpuscles
95	...	32	...	0·336
96	...	40	...	0·416
96	...	40	...	0·416
92	...	36	...	0·391
98	...	35	...	0·357
110	...	48	...	0·436
96	...	42	...	0·437
92	...	34	...	0·369

ANÆMIA

TABLE II

Hæmoglobin in 5 c. mm. of blood		Hæmoglobin in the Resistant Corpuscles in 5 c. mm. of blood		Relative Hæmo-globin-value of the Resistant Corpuscles
40	...	8	...	0·200
30	...	4	...	0·133
35	...	10	...	0·285
36	...	10	...	0·277

TABLE III

Hæmoglobin in 5 c. mm. of blood		Hæmoglobin in the Resistant Corpuscles in 5 c. mm. of blood		Relative Hæmo-globin-value of the Resistant erythrocytes
60	...	21	...	0·350
46	...	19	...	0·413
30	...	14	...	0·467
36	...	15	...	0·417

TABLE IV

Hæmoglobin in 5 c. mm. of blood		Hæmoglobin in the Resistant Corpuscles in 5 c. mm. of blood		Relative Hæmo-globin-value of the Resistant erythrocytes
35	...	20	...	0·571
42	...	23	...	0·547

It will be seen from the above tables that while in health the relative hæmoglobin-value of the erythrocytes varies within small limits, in anæmia it varies within much wider limits. Thus, in some cases, it is much below the normal, in others it is almost the same as normal, while in others again it is above the normal. In kala-azar it is generally below the normal, while in ankylostomiasis it is above the normal. The forms of anæmia in which this value is increased or diminished and its clinical significance can only be determined by further investigation.

Permeability of the Erythrocytes to Water as a Factor in the Production of Hæmolysis

An explanation may here be offered as to the cause of the differences of the hæmoglobin-value of the resistant corpuscles in health and disease. It is possible that the resistant corpuscles are less permeable to water or can bear the tension of distension better than those that hæmolyse. This permeability or the power of resisting rupture, is probably altered in anæmia, being increased in some and diminished in others, while in others again it remains normal.

The entrance of water into the erythrocytes may, therefore, to some extent, be dependent upon their specific permeability, and this may be independent of the force of osmosis. So, also, their power of resisting rupture from distension after the entrance of water into their substance may vary in the different erythrocytes. That these are important factors in the phenomenon of hæmolysis is borne out by the following facts :—If one part of human blood is mixed with one part of $N/10$ saline solution and then treated with two parts of $N/30$ saline, we find that the amount of hæmolysis is much greater than when the $N/10$ saline contains one per cent formol. The presence of formol cannot in any way change the concentration of the salts in the corpuscles, and its action must result either in increasing the resistance of the erythrocytes to rupture from osmotic distension or diminishing the permeability of water. Similarly, again, when blood is allowed to crenate between the slides for twenty-four hours or more, and then treated with $N/10$ saline solution, we find that some of them still remain crenated. Now, if crenation were simply due to osmosis, then the corpuscles would swell up and lose their crenation by re-absorption of water when treated with $N/10$ saline solution. The fact that some of them do not lose their crenation shows that they

have become less permeable to water. In other words, along with crenation the outer portion of the erythrocytes undergo some changes, as a result of which they do not allow the free passage of water into their structure by the process of osmosis. The same is also borne out by the fact that crenated corpuscles, sometimes occur in the blood in some forms of anæmia. This cannot be due to any stronger concentration of saline in the serum, as in anæmia the chlorides are not much appreciably increased in the serum.

	1	2	3	4	5	6	7	8
Salinity of Normal Serum	0·6435%	0·5850%	0·6435%	0·7020%	0·7020%	0·6435%	0·7020%	0·7020%

Average = 0·6654%

	1	2	3	4	5	6
Salinity of Serum in Anæmia	0·7605%	0·5850%	0·6727%	0·6727%	0·6435%	0·8772%

Average = 0·7019%

THE LAW REGULATING HÆMOLYSIS OF ERYTHROCYTES IN HYPOSMOTIC SALINE SOLUTION OR DISTILLED WATER

In studying the hæmoglobin-value of the resistant ery-throcytes when blood is treated with hyposmotic saline or distilled water, I have obtained results which lead to the conclusion that the amount of hæmoglobin in a suspension of erythrocytes that can be dissolved in a definite volume of hyposmotic saline solution or distilled water is proportional to the total amount of hæmoglobin present in the suspended corpuscles. The suspension of the erythrocytes was obtained by taking a volume of blood, which was allowed to clot after being quickly centrifuged. The clot was separated and the erythrocytes washed three or four times with 0.85 per cent solution of sodium chloride, and a suspension of the erythrocytes obtained. One volume of this was treated with two volumes of distilled water or $N/50$ sodium chloride solution. The amount of hæmoglobin dissolved was then estimated and compared with the total amount of hæmoglobin present in the suspended corpuscles. The blood of various animals was thus tested and the results compared.

In the following tables, t denotes the total amount of hæmoglobin in a suspension of erythrocytes, d the amount of dissolved hæmoglobin in any particular suspension of erythrocytes, and $p = \dfrac{d}{t}$:

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TABLE I (a)

Normal Human Blood (1 part of erythrocytes suspended in 0·85% NaCl solution + 2 parts of N/50 NaCl solution)—

1. $t = 110$; $d = 62$. $p \times 10^2 = 56\cdot3$
2. $t = 100$; $d = 58$. $p \times 10^2 = 58\cdot00$
3. $t = 90$; $d = 50$. $p \times 10^2 = 55\cdot55$
4. $t = 77$; $d = 44$. $p \times 10^2 = 57\cdot14$

TABLE I (b)

Normal Human Blood (1 part of erythrocytes suspended in 0·85% NaCl solution + 2 parts of distilled water)—

1. $t = 150$; $d = 106$. $p \times 10^2 = 77\cdot66$
2. $t = 118$; $d = 90$. $p \times 10^2 = 76\cdot27$
3. $t = 50$; $d = 35$. $p \times 10^2 = 70\cdot00$

TABLE II

Fowl's Blood (1 part of erythrocytes suspended in 0·85% NaCl solution + 2 parts of distilled water)—

1. $t = 105$; $d = 88$. $p \times 10^2 = 83\cdot80$
2. $t = 96$; $d = 78$. $p \times 10^2 = 81\cdot25$
3. $t = 90$; $d = 80$. $p \times 10^2 = 88\cdot88$
4. $t = 84$; $d = 68$. $p \times 10^2 = 80\cdot95$
5. $t = 42$; $d = 34$. $p \times 10^2 = 80\cdot95$

TABLE III (a)

Sheep's Blood (1 part of erythrocytes suspended in the serum of the sheep + 2 parts of distilled water)—

1. $t = 120$; $d = 120$. $p \times 10^2 = 100\cdot00$
2. $t = 70$; $d = 70$. $p \times 10^2 = 100\cdot00$

TABLE III (b)

Sheep's Blood (1 part of erythrocytes suspended in 0·85% NaCl solution + 2 parts of distilled water)—

1. $t = 150$; $d = 150$. $p \times 10^2 = 100\cdot00$
2. $t = 70$; $d = 70$. $p \times 10^2 = 100\cdot00$

TABLE IV (a)

Frog's Blood (1 part of erythrocytes suspended in the serum of the frog + 2 parts of N/50 NaCl)—

1. $t = 46$; $d = 10$. $p \times 10^2 = 20\cdot87$
2. $t = 28$; $d = 8$. $p \times 10^2 = 28\cdot57$

TABLE IV (b)

Frog's Blood (1 part of erythrocytes suspended in 0·85% NaCl solution + 2 parts of distilled water)—

    1. $t = 90$; $d = 10$. $p \times 10^2 = 11 \cdot 11$

    2. $t = 70$; $d = 8$. $p \times 10^2 = 11 \cdot 42$

TABLE V

Cat's Blood (1 part of erythrocytes suspended in 0·85% NaCl solution + 2 parts of distilled water)—

    1. $t = 122$; $d = 106$. $p \times 10^2 = 86 \cdot 88$

    2. $t = 62$; $d = 55$. $p \times 10^2 = 88 \cdot 71$

TABLE VI

Value of $p \times 10^2$ in the blood of a number of healthy students, the blood being treated with 2 parts of N/50 NaCl (deduced from the author's paper in the *Bio-Chemical Journal*, Vol. IV, Nos. 5, 6, and 7)—

1	2	3	4	5	6
58·4	58·4	60·9	64·3	56·4	56·3

From the above observations the following laws can be deduced :—

1. The amount of hæmoglobin dissolved in a given volume of hyposmotic sodium chloride solution or distilled water is proportional to the amount of hæmoglobin in the erythrocytes presented for solution in the form of a suspension.

2. The ratio between these above two factors varies in different animals, being the smallest in the case of the frog, and greatest in the case of the sheep. (In the case of the rabbit it is also very high.)

3. It is fairly constant in healthy individuals, but varies widely in disease. [1]

From the above it will be seen that the same law that regulates the solution of globulin in solution of neutral salts

[1] *Bio-Chemical Journal*, Vol. IV, 1909, p. 280.

also regulates the solution of hæmoglobin contained within erythrocytes. In other words, the latter follows the law enunciated by Mellanby and Hardy.[1] This fact probably throws some light as to how hæmoglobin exists inside erythrocytes. Each individual erythrocyte allows a certain quantity of water to enter into its substance by process of osmosis when blood is treated with a hyposmotic sodium chloride solution or distilled water. If we now consider that the hæmoglobin exists in the form of a suspension inside erythrocytes, it follows, from the law of Mellanby and Hardy, that the amount of hæmoglobin that will be dissolved by water entering into the substance of the erythrocytes is proportional to the amount of hæmoglobin in each individual corpuscle. Accordingly, the amount of dissolved hæmoglobin that will pass out of the corpuscles is proportional to the amount of hæmoglobin inside them. It follows, therefore, that the amount of hæmoglobin dissolved in a suspension of erythrocytes when the latter is treated with distilled water or hyposmotic saline, is proportional to the total amount of hæmoglobin present in them. We can, therefore, conclude that hæmoglobin as it exists inside erythrocytes is in a state of suspension.

[1] *Journal of Physiology*, Vol. XXXIII, 1905; also referred to in the *Proceedings* of the Royal Society, Series B, Vol. LXXIX, 1907.

ON THE FREEZING POINT OF THE UN-HÆMOLYSED CORPUSCLES DURING HÆMOLYSIS OF BLOOD AND ON THE EFFECTS OF EVAPORATION ON THE RESISTANCE OF ERYTHROCYTES TO HÆMOLYSIS

In a previous communication (*Bio-Chem Jour.*, Vol. IV) I have pointed out that the resistant corpuscles during hæmolysis of blood are either less permeable to water or can bear the tension of distension better than those that hæmolyse.

In order to determine whether the unhæmolysed corpuscles are less permeable to water or not, I have estimated the freezing point of the unhæmolysed corpuscles as well as that of the supernatant fluid after the separation of the unhæmolysed corpuscles by centrifugalisation.

In all of my experiments the blood was allowed to clot, the clotted blood squeezed through thin muslin and mixed with two parts of distilled water, and the mixture thoroughly centrifuged after one hour.

The following results have been obtained : —

(1) HUMAN BLOOD.   (Blood taken from the jugular veins four hours after death)

1.	$\triangle$ for the hæmolysed corpuscles	...	...	...	0·195
2.	$\triangle$ for the unhæmolysed corpuscles	...	...	...	0·195

(2) FOWL'S BLOOD

1.	$\triangle$ for the hæmolysed corpuscles	...	...	...	0·224
2.	$\triangle$ for the unhæmolysed corpuscles	...	...	...	0·285

(3) Fowl's Blood

1.	△ for the hæmolysed corpuscles	...	...	... 0·210
2.	△ for the unhæmolysed corpuscles	...	...	... 0·210

(4) Fowl's Blood

1.	△ for the hæmolysed corpuscles	...	...	... 0·210
2.	△ for the unhæmolysed corpuscles	...	...	... 0·200

(5) Fowl's Blood

1.	△ for the hæmolysed corpuscles	...	...	... 0·245
2.	△ for the unhæmolysed corpuscles	...	...	... 0·208

(6) Fowl's Blood

1.	△ for the hæmolysed corpuscles	...	...	... 0·230
2.	△ for the unhæmolysed corpuscles	...	..	... 0·190

(7) Fowl's Blood

1.	△ for the hæmolysed corpuscles	...	...	.. 0·250
2.	△ for the unhæmolysed corpuscles	...	...	... 0·250

It will be seen from the above that the freezing points of the hæmolysed corpuscles were nearly the same as those of the unhæmolysed corpuscles, and therefore osmosis must have taken place to the same extent in the hæmolysed as well as the unhæmolysed corpuscles. In other words, the unhæmolysed corpuscles are nearly as much permeable to water as the hæmolysed ones. The fact that some of the corpuscles do not hæmolyse must, therefore, be due to their being able to bear the tension of distension better than those that hæmolyse. We may describe this resistance of the erythrocytes to rupture as their specific resistance. This specific resistance to rupture varies in different animals, and may be expressed by the relative hæmoglobin value of their resistant erythrocytes.

In the following tables the erythrocytes were invariably suspended in 0·85 per cent saline, and the amount of

suspension taken was always half that of the dissolving fluid, which in the present case was distilled water :—

Specific Resistance of Erythrocytes to Rupture in Different Animals

1.	Human blood	...	...	...	...	... 0·2934
2.	Fowl's blood	...	...	...	...	... 0·1875
3.	Dog's blood	...	...	..	...	... 0·3333
4.	Frog's blood	...	...	...	...	... 0·7917
5.	Sheep's blood	...	...	...	...	... 0
6.	Rabbit's blood	...	..	...	...	... 0

Effect of Evaporation on the Resistance of Erythrocytes to Hæmolysis

If we spread the erythrocytes, from a suspension in N/10 sodium chloride solution, over a slide and then allow them to dry gently, we find that these dried corpuscles when treated with N/10 sodium chloride solution completely dissolve.   Let us consider what happens when the erythrocytes are drying on the slide.   They tend to stick to the slide and, as evaporation goes on, they tend to contract.   As a result of these two antagonistic processes there is, perhaps, rupture of their membranous structure, and they dissolve when they come in contact with what would be an isotonic solution in the case of undried erythrocytes.   Their complete solution indicates complete disruption of their cellular framework in the process of drying up.

This complete disruption is, as just now stated, either purely mechanical due to rupture of all the membranous portions inside the erythrocytes that become adherent to the slide, or the removal of water during evaporation brings about a complete change in the chemical constitution of the erythrocytes, converting them into particles soluble in a saline of any strength, just as a lump of sugar dissolves in water. This latter view best explains the complete solution of

erythrocytes when treated with saturated solution of sodium chloride in water. In the process of desiccation of the erythrocytes by the saturated sodium chloride solution, water is removed from them. A portion of this water is perhaps in chemical combination with the erythrocytes, and this compound is broken up when they are allowed to dry up over a slide or are treated with saturated sodium chloride solution. Water, therefore, as it exists inside erythrocytes, is partly in a chemical combination with its structure, and this compound may be expressed as $X\big<^{H}_{OH}$.

AN INVESTIGATION INTO THE PHYSICO-CHEMICAL MECHANISM OF HÆMOLYSIS BY SPECIFIC HÆMOLYSINS (PRELIMINARY COMMUNICATION

The mechanism of hæmolysis by specific hæmolysins has not been much investigated from the physico-chemical standpoint. It is supposed that the hæmolysin affects the permeability of the envelope. Baumgarten has noticed that the first stage of hæmolysis, as produced by a biological poison, is the swelling of the corpuscles, just as occurs when the serum is made hypotonic. Bang (1910) considers that hæmolysis by means of cobra poison is due to change in the composition of the lipoid membrane of the erythrocyte rendering it more permeable to salts. Through this membrane the extracellular salts and water soak gradually, until in the end the corpuscle ruptures. The behaviour of the nucleated erythrocytes of amphibians towards specific hæmolysins tends to show that their permeability is alone affected during the process [Landau, 1903].

So far as I am aware, no work has been done on the behaviour of erythrocytes loaded with amboceptor towards distilled water before the complement has been allowed to act upon them. The present paper gives the results of investigations on the determination of the resisting power of amboceptor-loaded human erythrocytes to hæmolysis when under the influence of distilled water. The hæmolytic antisera were obtained from fowls and rabbits that had been immunised by means of injections of washed human corpuscles.

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It was originally expected that amboceptor-loaded corpuscles would be less resistant than normal ones in their behaviour towards distilled water. Contrary to what was expected, I noticed a remarkable increase of the resisting power of the erythrocytes.

The method adopted for determining the resisting power of the erythrocytes is that previously described by me [1909, 1911] for the determination of the specific resistance of erythrocytes to hæmolysis. A series of experiments were performed and these are described as follows :—

In the first series, the erythrocytes were suspended in 0˙85 per cent NaCl and allowed to hæmolyse with two parts of distilled water. The mixture was allowed to stand for ten minutes and the dissolved hæmoglobin was estimated in the manner previously described. This gave the resistance of the normal erythrocytes to hæmolysis.

In the second series, the erythrocytes were mixed with two parts' of a 10 per cent dilution of complement-free anti-human fowl's hæmolytic serum with 0˙85 per cent NaCl and kept in the incubator for one to three hours and subsequently treated in the same way as above with distilled water after having been thoroughly washed in 0˙85 per cent NaCl, and the resistance of the amboceptor-loaded erythrocytes determined in the same way as above.

In the third series, the erythrocytes were treated with complement-free antihuman rabbit's hæmolytic serum and their resistance to hæmolysis determined.

As a control test, a few experiments were made to determine the resistance of human erythrocytes after having been treated with normal fowl's serum in the same way as above.

In this way the resistance of the erythrocytes under different conditions was determined and the following tables worked out. The resistance of the erythrocytes is expressed in terms of the relative hæmoglobin value of the resistant

erythrocytes which is the ratio between the amount of hæmoglobin in the resistant corpuscles and the amount in the total suspension of erythrocyte used.

Tables showing the resistance of erythrocytes to hæmolysis under different conditions :—

TABLE I

	Resistance of normal erythrocytes to hæmolysis	Resistance of erythrocytes loaded with amboceptor obtained from fowl
1	0·345	0·500
2	0·379	0·456
3	0·242	0·407
4	0·333	0·480
5	0·346	0·452
6	0·269	0·531
7	0·333	0·466
8	0·350	0·444
9	0·301	0·421
10	0·288	0·459
11	0 288	0·368

TABLE II

	Resistance of normal erythrocytes to hæmolysis	Resistance of erythrocytes loaded with amboceptor obtained from rabbits
1	0·195	0·600
2	0·188	0·378
3	0·296	0·592
4	0·240	0·480
5	0·132	0·220

TABLE III

	Resistance of normal erythrocytes to hæmolysis	Resistance of erythrocytes treated with normal fowl's serum
1	0·288	0·243
2	0·288	0·298
3	0·282	0·259
4	0·240	0·282

It will be seen from the above tables that in every case the addition of the amboceptor increased the specific resistance of erythrocytes, while the addition of normal fowl's serum did not bring about any difference in their resisting power.

The problem that we are now to solve is how it is that erythrocytes become more resistant when they become fixed to the amboceptor but lose all their resisting power as soon as the complement acts and what is the biological significance of this remarkable phenomenon. In the first process of the action of hæmolysin during which the amboceptor combines with the erythrocytes we may assume that this combination is purely of the nature of *adsorption* and not true chemical combination. In this process the dimensions of the pores between the molecules of the outer wall of the erythrocytes become small due to the amboceptor molecules filling the pores of the original erythrocytes. As diffusion depends specially upon the dimensions of the pores of the membrane through which it takes place, less hæmoglobin passes out through the red corpuscles loaded with amboceptor than the unloaded ones when they are treated with distilled water. In the case of the colloidal complex of amboceptor and erythrocyte molecules, the bodies brought into close contact with each other do not react with

one another in the chemical sense. Chemical reaction which, according to Bayliss [1912], is the third and last stage of th e heterogeneous reactions of colloidal complexes, takes place between the molecules of the amboceptor and of the erythrocytes only through the agency of the complement, which probably acts more or less like a ferment. When this takes place, there is a condensation of the molecules of the colloidal complex in each erythrocyte and as a result of this, the dimensions of the pores of the membranes of the red corpuscles increase to such an extent that hæmoglobin diffuses out of the corpuscles and hæmolysis results.

This theory will easily explain the inhibitory influence exerted by hypertonic saline solutions on the action of specific hæmolysins as has been observed by Sutherland and McCay [1911] and others. Such saline solutions would tend to reduce the size of the erythrocytes by exosmosis of water from them and as a result of this, the molecules of the erythrocytes come closer to each other and hæmolysis is prevented. In other words, the widening of the pores of the erythrocytes brought about by the action of the complement on the amboceptor-loaded erythrocytes is counteracted by the presence of the hypertonic saline.

To test the accuracy of the theory the following experiments were performed :—

A suspension of sheep's corpuscles in normal saline was mixed with amboceptor and complement and kept in the incubator. The process of hæmolysis was stopped in from five to ten minutes before it was complete, the corpuscles quickly centrifuged and the supernatant fluid replaced by N/2 Nacl solution, after thoroughly washing the corpuscles several times with the same. A portion of the suspension of the corpuscles in N/2NaCl was again centrifuged and the N/2 solution was pipetted off and in its place an excess of 0·85 per cent NaCl solution was

substituted—a marked hæmolysis resulted. Thus we have the following results :--

(1) Amboceptor-loaded corpuscles partially acted upon by the complement, thoroughly washed in N/2NaCl—no hæmolysis.

(2) Amboceptor-loaded corpuscles partially acted upon by the complement, thoroughly washed in N/2NaCl and then the N/2NaCl replaced by 0·85 per cent NaCl — distinct hæmolysis.

In other words, amboceptor-loaded corpuscles which have been partially acted upon by the complement are hæmolysed when brought in contact with normal saline. The dimensions of the pores of such corpuscles when suspended in N/2NaCl are much less than when suspended in normal saline, i.e., there is marked widening of their pores when they are suspended in normal saline and hæmoglobin diffuses out. Evidently, therefore, the pores of the amboceptor-loaded corpuscles that have been acted upon partially by the complement are much wider than those of the normal ones. The complement must have, therefore, brought about a condensation of the molecules of the amboceptor-loaded corpuscles, as explained before, thereby leading to widening of their pores to such an extent as to allow the hæmoglobin molecules to pass out of them, even when they are suspended in normal saline.

An attempt was made to determine the volume of the corpuscles before and after combination with amboceptor. After rejecting different methods, the following method was devised for determining the size of the corpuscles. Human and sheep's corpuscles were used for the purpose.

The corpuscles were first suspended in 0·85 per cent NaCl and the suspension was sucked into two large-bore hæmatocrit tubes of exactly the same diameter and the corpuscles in both were centrifugalised at a high speed for about ten minutes, after which their volume was noted. The clear supernatant

fluid from one of the tubes was now pipetted off and a dilution of hæmolytic amboceptor in 0˙85 per cent NaCl (one part of amboceptor and nine parts of 0˙85 per cent NaCl) introduced in its place. The amboceptor was then thoroughly mixed with the corpuscles and the mixture allowed to stand for ten minutes. The other tube which was used as a control was then shaken up and the corpuscles again mixed with the supernatant fluid. The two tubes were again centrifugalised for ten minutes and the volume of the corpuscles noted. If the volume of the corpuscles in the control tube was found to be exactly equal to what was noted in it in the first experiment, then there was no error due to any change in the speed of the centrifuging tube. The volume of the amboceptor-loaded corpuscles was then noted. If the volume of the corpuscles in the control tube was different in the second experiment from what was noted in the first, then the volume of the amboceptor-loaded corpuscles was accordingly changed. In this way, absolutely, accurate results were obtained.

Thus if V be the volume of the corpuscles in the control tube after the first centrifuging and V' after the second, then $V - V'$ is the difference due to change in the speed of the centrifuging machine.

If now v be the volume of the corpuscles before being loaded with amboceptor, and v' after being loaded with amboceptor, it is evident that $v - v'$ is not actually the change in the volume of the corpuscles. The exact change is, evidently,

$$(v - v') + \frac{v}{V}\left(V - V'\right).$$

In this way, the exact change in the volume of the

corpuscles due to combination with amboceptor was found and the following results were obtained :—

Human corpuscles

Volume before action of amboceptor		Volume after action of amboceptor	
1	2·75 c. mm.	1	2·75 c. mm.
2	2·75	2	2·75
3	2·50	3	2·50
4	1·50	4	1·50
5	1·45	5	1 50

Sheep's corpuscles

Volume before action of amboceptor		Volume after action of amboceptor	
1	3·30 c. mm.	1	3·30 c. mm.
2	5·38	2	5·20
3	4·50	3	4·20
4	6·23	4	6·22

It will be seen from the above tables that there was no reduction in the volume of the corpuscles after the action of the amboceptor and therefore the increase in their resistance to hæmolysis was not due to any diminution in their volume after the action of the amboceptor.

I am deeply indebted to Lt.-Col. Sutherland, I.M.S., of the Calcutta Medical College, for kindly providing me with the hæmolytic antisera and giving me every facility to work in his laboratory.

References

Bang (1910), *Bio-Chem. Zeitsch.*, **23**, 463.
Bayliss (1912), *Proc. Roy. Soc.*, B, **84**, 81.
Brahmachari (1909), *Bio-Chem. J.*, **4**, 59, 280.
—— (1911), *Bio-Chem. J.*, **5**, 291. (See also *Studies in Hæmolysis*, 2nd Ed.)
Landau (1903), *Ann. Inst. Past.*, **17**, 52.
Sutherland and McCay (1911), *Bio-Chem. J.*, **5**, 1.

AN INVESTIGATION INTO THE PHYSICO-CHEMICAL MECHANISM OF HÆMOLYSIS BY SPECIFIC HÆMOLYSINS. NO. II. THE ELECTRICAL CONDUCTIVITY OF SENSITISED CORPUSCLES AND THE ACTION OF INORGANIC FERMENTS OR METAL-SOLS UPON THEM

In my previous communication [1913] I pointed out that in the process of hæmolysis by specific hæmolysins, the amboceptor molecules are at first adsorbed into the pores in the walls of the erythrocytes and subsequently a true chemical combination takes place between these and the molecules of the corpuscular walls.

Investigations have since been carried on to determine the electrical conductivity of the sensitised corpuscles as compared with the unsensitised ones. The following methods were adopted:—Sheep's corpuscles after being washed with normal saline were treated with six times the working dose of anti-sheep amboceptor and the mixture kept for a variable period, from two to twenty-four hours, in the ice-chamber. Subsequently the corpuscles were centrifuged and thoroughly washed with normal saline. In a second series of observations the sensitised as well as the unsensitised corpuscles were suspended in 5 per cent glucose solution and then separated by centrifugalistion. The conductivity of the corpuscles was measured by

Kohlrausch's method and the following results were obtained. In all cases the conductivity was calculated at 26°C.

TABLE I

The electrical conductivity of the sediment of corpuscles from the emulsion in normal saline

(a) $\begin{cases} \text{(1) Unsensitised corpuscles} & K(26°\text{C.}) \times 10^5 = 360 \\ \text{(2) Sensitised corpuscles} & K(26°\text{C.}) \times 10^5 = 300 \end{cases}$

(b) $\begin{cases} \text{(1) Unsensitised corpuscles} & K(26°\text{C}) \times 10^5 = 300 \\ \text{(2) Sensitised corpuscles} & K(26°\text{C}) \times 10^5 = 120 \end{cases}$

(c) $\begin{cases} \text{(1) Unsensitised corpuscles} & K(26°\text{C.}) \times 10^5 = 190 \\ \text{(2) Sensitised corpuscles} & K(26°\text{C.}) \times 10^5 = 160 \end{cases}$

TABLE II

The electrical conductivity of the sediment of corpuscles from the emulsion in 5% glucose solution in distilled water

(a) $\begin{cases} \text{(1) Unsensitised corpuscles} & K(26°\text{C}) \times 10^5 = 250 \\ \text{(2) Sensitised corpuscles} & K(26°\text{C.}) \times 10^5 = 100 \end{cases}$

(b) $\begin{cases} \text{(1) Unsensitised corpuscles} & K(26°\text{C.}) \times 10^5 = 70 \\ \text{(2) Sensitised corpuscles} & K(26°\text{C.}) \times 10^5 = 38 \end{cases}$

It will be seen from the above tables that the adsorption of amboceptor by erythrocytes was followed by a diminution of the electrical conductivity of the corpuscles and this was specially marked when the sediment of corpuscles was obtained from an emulsion in isotonic glucose solution.

In the case of the sediment from the suspension in normal saline, the conductivity is mainly due to the ions still in contact with the corpuscular walls. Therefore, the corpuscular walls of the sensitised corpuscles obstruct the passage of the ions more than the unsensitised ones. In the case of the sediment from glucose solution, the conductivity is mostly due to the envelopes. From these it may be

concluded that the walls of the sensitised corpuscles conduct electricity worse than those of the unsensitised ones.

From the above it may be assumed that the diminution of conductivity of the corpuscular walls in the case of sensitised corpuscles is due to the action of amboceptor on the walls and therefore that at least a portion of the amboceptor is in some sort of combination with the corpuscular walls in sensitised corpuscles.

Action of Inorganic Ferments or Metal-sols upon Sensitised Corpuscles

A series of experiments were undertaken to determine the action of catalysts, such as animal charcoal and platinum black, upon sensitised corpuscles. No hæmolysis was brought about by their action. Colloidal solution of iodine (Collo-Iode of Dubois) also gave negative results. Various metal-sols were mixed in varying concentration with the sensitised corpuscles. The following have, up to the present time, been used : Electrargol and electro-selenium prepared by Clin and Co. (Paris), collo-sol silver and collo-sol mercury prepared by Oppenheimer and Sons.

Up to now, all such experiments with colloids have failed to bring about hæmolysis of the sensitised corpuscles.

CONCLUSIONS

(1) In the process of adsorption of amboceptor by the erythrocytes, it is in the corpuscular walls that the molecules of the amboceptor remain adsorbed.

(2) The electrical conductivity of the corpuscular walls of the amboceptor-loaded corpuscles is less than that of normal erythrocytes.

(3) Metal-sols, as well as other catalysts, such as animal charcoal, platinum black, colloidal iodine, do not bring about any hæmolysis of sensitised corpuscles.

I am deeply indebted to Col. Sutherland, I. M. S., and Dr. G. C. Mitter of the Medical College, Calcutta, for providing me with the hæmolytic antisera for my experiments, and to Professor Nilratan Dhar of the Presidency College, Calcutta, for assisting me in determining the electrical conductivities.

Reference

Brahmachari, U. N. (1913), *Bio-Chem. J.* 7, 562.

BEHAVIOUR OF THE ERYTHROCYTES TOWARDS HYPEROSMOTIC SOLUTIONS OF NaCl—THE CURVES OF HÆMOLYSIS WITH HYPEROSMOTIC NaCl SOLUTIONS —THE PROPERTIES OF SALTED ERYTHROCYTES

Behaviour towards very concentrated NaCl solutions :—
When human blood is mixed with 2 volumes of a saturated NaCl solution in distilled water (which we shall henceforth call $\frac{S}{1}$ NaCl sol.), the mixture at once becomes turbid. The turbidity is followed within a few minutes by a marked solution of the erythrocytes and the mixture at the same time becomes clear to a great extent. In the rabbit's blood no such clearing up of the mixture takes place within a short time and the fluid remains turbid for a much longer time. If the rabbit's blood after it has been treated in the above way be centrifugalised within ten minutes, it is found that the supernatant liquid becomes faintly red, showing that only slight hæmolysis had taken place, contrary to what is found in the case of human blood in which the supernatant fluid is found to be markedly red.

Similarly, if one part of human blood is mixed with two parts of $\frac{S}{2}$*, $\frac{S}{3}$, up to $\frac{S}{7}$ NaCl solution a similar turbidity is noticed, but at first no hæmolysis is observed within a few minutes. If the sediment obtained, after centrifuging

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* $\frac{S}{1}$ or saturated NaCl solution $=6\frac{N}{1}$ NaCl nearly at the temperature at which we have worked; $\frac{S}{2}$=one part of a saturated NaCl solution diluted with one part of distilled water; $\frac{S}{3}$=one part of a saturated NaCl solution diluted with two parts of distilled water, and so on.

each of the above mixtures, be mixed with the supernatant fluid at its top, we find that the sediment dissolves more and more, each time the process is repeated, making the supernatant fluid more and more red; finally a sediment remains behind which does not dissolve any more and which is smaller in amount the greater the strength of the NaCl solution. With $\frac{S}{35}$ we find generally no hæmolysis takes place.

The sediment from each of the above mixtures also possesses the remarkable property of being dissolved in $\frac{N}{10}$ NaCl solution, the amount dissolved varying with the strength of the NaCl with which the blood was previously treated. Thus the sediment from the mixture containing $\frac{S}{1}$ NaCl dissolves completely, while that containing $\frac{S}{7}$ dissolves only partially in the $\frac{N}{10}$ NaCl solution. It is thus seen while $\frac{N}{10}$ NaCl acts as an *inactive* fluid towards the sediment of a mixture of blood and two vols. of a hyposmotic NaCl solution, it has the property of dissolving completely the sediment from blood which has been previously treated with two vols. of $\frac{S}{1}$ NaCl solution.

The amount of hæmolysis obtained by mixing blood with the concentrated NaCl solution increases with the extent of time the blood is kept mixed with the solution. Thus, when human blood is mixed with $\frac{S}{1}$ NaCl solution, the supernatant fluid at first appears colourless, but after an hour some hæmolysis is observed in the mixture.

From the above it will be seen that in describing the curve of hæmolysis in human blood treated with hyperosmotic NaCl solutions, we have to take the following two factors into consideration,—(1) effect of time and (2) effect of repeated centrifugalisations. In this way three curves can be described :—(1) Curve of hæmolysis in human blood treated with varying dilutions of $\frac{S}{1}$ NaCl solution and the mixture very quickly centrifugalised. (2) Curves of hæmolysis after repeated centrifugalisations. (3) Curve of hæmolysis in the mixture after sometime (say an hour).
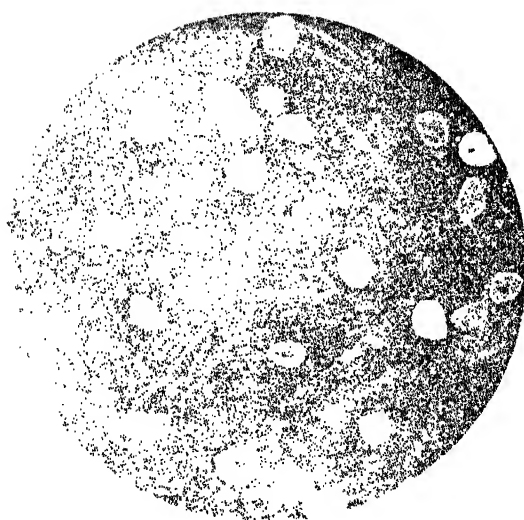
The salted erythrocytes of the rabbit obtained by centrifuging a mixture of one part of blood and two parts of saturated NaCl solution.
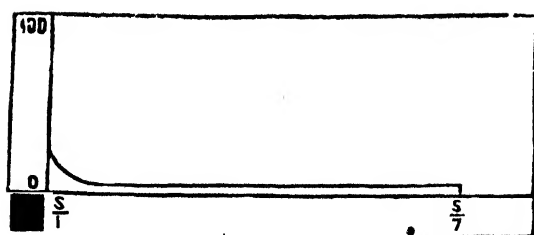
Curve of hæmolysis with hyperosmotic NaCl solution, the mixture being very quickly centrifuged (one part of human blood + two parts of NaCl solution of strengths between $\frac{S}{1}$ and $\frac{S}{7}$).
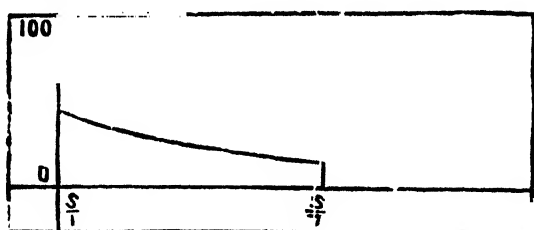
Curve of hæmolysis with hyperosmotic NaCl solution, after repeated centrifugalisation of the mixture (one part of blood + two parts of NaCl solution of strengths between $\frac{S}{1}$ and $\frac{S}{7}$).
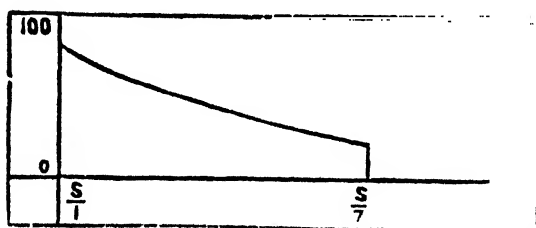
Curve of hæmolysis with hyperosmotic NaCl solution, one hour after mixture of the blood with the NaCl solution (one part of blood + two parts of NaCl solution of strengths between $\frac{S}{1}$ and $\frac{S}{7}$).

The salted erythrocytes :—By salted erythrocytes, I mean, the erythrocytes that are found in the sediment after centrifugalisation of a mixture of blood and a very hyperosmotic NaCl solution.

The behaviour of human blood towards saturated NaCl solution has been already described. We now proceed to describe the properties of the salted erythrocytes. As the erythrocytes of the rabbit's blood show these properties very distinctly and as

they have not been studied by any previous observer, I shall describe them here at some length.   The erythrocytes of human blood also possess the same properties to a more or less extent.

(1)   If the sediment of the rabbit's blood obtained in the above way be mixed with the supernatant fluid at its top, it acquires the remarkable property of being dissolved to some extent, showing, as it were, that the hæmoglobin was squeezed out of the erythrocytes during the process of centrifugalisation.   This phenomenon was discovered in the following way :—Rabbit's blood was mixed with 2 volumes of a saturated NaCl solution in two separate tubes ; one of the mixtures was then centrifugalised and the other one left undisturbed.   The sediment in the centrifugalised blood was mixed with the supernatant fluid and the mixture was again centrifugalised and so on.   It was found that each time the sediment was mixed with the supernatant fluid after each successive centrifugalisation and then the mixture • centrifugalised again, the tint of the supernatant fluid become more and more red.   On the other hand, when the mixture in the other tube was centrifugalised after quarter of an hour during which the former experiment was performed, it shewed very much less red tint at the top than in the supernatant fluid in the other tube.   From this it is evident that the salted erythrocytes undergo hæmolysis each time they are centrifugalised, the hæmoglobin being, as it were, squeezed out of them in their vibration during centrifugalisation and also when they are jammed against each other at the bottom of the centrifuge tube.

(2) The corpuscles in the same sediment dissolve to a very great extent when treated with $\frac{N}{10}$ NaCl solution.

(3) The salted erythrocytes are found to be contracted in size but many of them present a globular shape and do not appear wrinkled or crenated, as one would expect to observe from the membrane-theory of the walls of the erythrocytes.   Some of them still contain hæmoglo-

bin, while others are decolourised. Many of those that are decolourised present a globular appearance under the microscope. That they are contracted is shown by the following table :—

	Volume of normal red corpuscles to total blood.	Volume of the salted red corpuscles to total blood. (Blood+2 vols. of $\frac{S}{2}$ NaCl.)
Rabbit	$\frac{51}{100} = 0\cdot51$	$\frac{5}{18} = 0\cdot28$
Human	$\frac{43}{88} = 0\cdot51$	$\frac{3}{10} = 0\cdot30$
Human	$\frac{15}{29} = 0\cdot52$	$\frac{5}{12} = 0\cdot42$
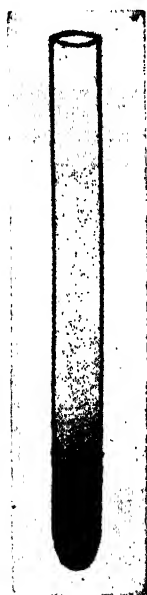
1.—The supernatant fluid obtained by centrifuging a mixture of one part of rabbit's blood and two parts of saturated NaCl solution within a quarter of an hour after the mixture was prepared—very faint red colou in the supernatant fluid.

2.—The sediment from the above mixed with the supernatant fluid—marked redness of the supernatant fluid after centrifugalisation.

The method by which I estimated the volume of the corpuscles in normal blood consisted in mixing a specimen

of the blood with a definite volume of $2\frac{1}{2}$ per cent solution of $K_2Cr_2O_7$, and calculating from this the volume of the corpuscles after centrifugalisation. In the case of the blood treated with $\frac{S}{1}$ or $\frac{S}{2}$ NaCl, the mixture was simply centrifuged and since no coagulation took place, the volume was easily calculated.

I would now summarise all the phenomena presented by the rabbit's blood when treated with a saturated NaCl solution :—

(1) Rabbit's blood $+\frac{S}{1}$ NaCl $=$ Red sediment on centrifugalisation, and at first a colourless supernatant fluid.

(2) The sediment from (1) + the supernatant fluid $=$ partial solution of the erythrocytes ; on repeated centrifuging and mixing the sediment with the supernatant fluid, more and more of the erythrocytes are dissolved.

(3) Sediment from (1) $+\frac{N}{10}$ NaCl solution $=$ well marked solution of the erythrocytes.

(4) Sediment from (1) shows erythrocytes much contracted in size, but many of them present a globular shape and are not wrinkled or crenated ; some contain hæmoglobin, and others are decolourised.

(5) Blood $+\frac{S}{1}$ NaCl solution $=$ a very turbid appearance ; this turbidity lasts longer in the case of the rabbit's than in the case of human blood ; at first the turbid fluid when centrifuged shows no red tint in the supernatant fluid in the case of rabbit's blood, but in the case of man, a red tint always appears. After an hour, the mixture of the rabbit's blood also shows a red tint in the supernatant fluid after centrifugalisation.

The most probable explanation of the above phenomena appears to me to be a marked change in the outer wall of the erythrocytes brought about by NaCl of the saturated solution ; probably a sort of combination takes place between NaCl and the outer walls of the erythrocytes. In the next chapter, I shall show the possibility of the presence

of such a compound. This compound finally leads to the destruction of some portion of the walls of the erythrocytes.

When blood is mixed with saturated NaCl solution, no doubt water comes out of the erythrocytes by the process of osmosis and they accordingly contract; when the sediment from the above is treated with $\frac{N}{10}$ NaCl, water re-enters their structure and as a result of this they try to expand and regain their original size. But either they burst before or as soon as they recover their original size or it may be that the water of the $\frac{N}{10}$ NaCl solution decomposes or dissolves the compound formed by NaCl with the outer wall of the erythrocytes. This compound is probably very easily decomposed.

It is evident that *osmosis* alone can explain the hæmolysis of blood by saturated NaCl solution. The remarkable phenomenon of hæmoglobin coming out of the corpuscles during centrifugalisation is probably explained by assuming that the damaged walls of the erythrocytes allow hæmoglobin to pass through them by a process allied to *filtration* under high pressure. As soon as there is decomposition of the unstable compound of the NaCl with the outer wall of the erythrocytes, the latter behave like small spheres of sponges containing dissolved colouring matter. Probably the same changes also take place in the erythrocytes when blood is treated with $\frac{S}{2}$ or half-saturated NaCl solution.
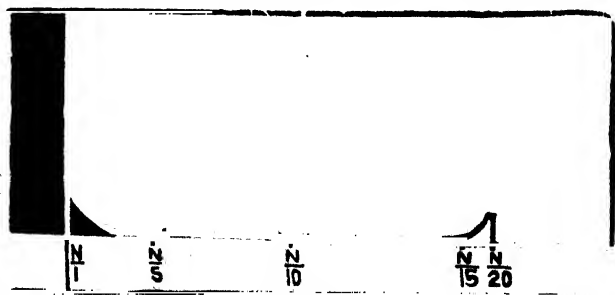
Curve of hæmolysis with NaCl solutions of strengths beween $\frac{N}{1}$ and $\frac{N}{20}$ (one part of human blood + two parts of NaCl solution).

Behaviour of the erythrocytes towards less concentrated solutions of NaCl

When human blood is treated with two volumes of a hypertonic NaCl solution of the strength $\frac{S}{7}$, we find that the mixture becomes somewhat opaque and bright red. As stated before, the sediment from such a mixture when mixed with the supernatant fluid shows some hæmolysis, but it is much less than when the blood is treated with saturated NaCl solution.

Behaviour of blood towards NaCl solutions of strengths between $\frac{S}{7}*$ and $\frac{N}{20}$

Between these limits we find that with $\frac{N}{5}$, generally no hæmolysis takes place, so also with $\frac{N}{10}$ and $\frac{N}{15}$ NaCl solutions.

According to my calculation, $\frac{S}{7}$ NaCl $= \frac{6}{7} \frac{N}{1}$ NaCl nearly.

POSSIBILITY OF ABSORPTION OF SODIUM CHLORIDE BY THE ERYTHROCYTES WHEN BLOOD IS TREATED WITH SATURATED OR HALF-SATURATED NaCl SOLUTION

The researches of Hedin[1], Eykman[2], Stewart[3], and others show that the permeability of the erythrocytes for sodium chloride is very slight or none at all. As far, however, as I am aware there are no records of any observations of the action of saturated or half-saturated NaCl solution upon erythrocytes.

I have shown in the previous chapter that the properties of salted erythrocytes would lead one to the supposition that a possible combination takes place between NaCl of the saturated or half-saturated NaCl solution and the erythrocytes. Whether any NaCl is absorbed or not by the erythrocytes when blood is treated with saturated or half-saturated NaCl solution can be determined in two ways :—

(1) By taking the electric conductivity of the blood before and after the mixture.

(2) By actual chemical analysis of the supernatant fluid after centrifugalisation of the mixture.

I have made some observations according to the second method of investigation and the few experiments, that I

[1] Pfluger's Archives, LXVIII, 1897.

[2] *Ibid*, S. 58.

[3] Journal of Physiology, Vol. XXVI, 1901.

have carried out show the great possibility of the absorption of NaCl by the erythrocytes under the circumstances mentioned above.

As this method of investigation is my own, I describe it in detail :—

This *chemical analysis method* depends upon the following calculations :

(1)   Volume of the serum in normal blood.

(2)   Volume of the serum in blood treated with $\frac{S}{1}$ or $\frac{S}{2}$ NaCl solution.

(3)   The quantitative estimation of the chlorides of the serum.

(4)   The quantitative estimation of NaCl in the solution of NaCl used, say $\frac{S}{2}$.

(5)   The quantitative estimation of the chlorides in the serum after blood is mixed with, say two volumes of $\frac{S}{2}$ NaCl solution.

(6)   The theoretical estimation of the chlorides in the above serum.

(7)   The presence of any difference between the above two estimations, *i.e.*, (5) and (6) and its explanation.

1st experiment.

Rabbit :

(1)   One part of rabbit's blood was taken and centrifuged after being mixed with $2\frac{1}{2}$ per cent solution of $K_2Cr_2O_7$. It was found by an average of 3 experiments :

$$\frac{\text{Volume of Serum}}{\text{Volume of Blood}} = \frac{51}{100} \text{ or } \tfrac{1}{2} \text{ nearly.} \qquad (1)$$

(2)   One volume of blood was mixed with 2 vols. of $\frac{S}{2}$ NaCl solution and the mixture was quickly centrifuged. It was found that $3\frac{3}{8}$ parts of the mixture contained $3\frac{1}{16}$ parts of supernatant fluid, *i.e.*, $\frac{49}{54}$ part of the supernatant fluid or diluted serum was contained in one part of the mixture.   (2)

(3) By quantitative estimation, the chlorides of the rabbit's serum were found as follows :—

$$1 \text{ vol. of serum} = \tfrac{3}{25} \text{ vol. of } \tfrac{N}{1} \text{ NaCl.} \tag{3}$$

(4) The quantitative estimation of the chlorides in the supernatant fluid after centrifugalisation of a mixture of 1 part of blood and 2 parts of $\tfrac{S}{2}$ NaCl solution was found to be, by an average of three experiments, as follows :—

$$1 \text{ vol. of the supernatant fluid} = 1\cdot833 \text{ vol. of } \tfrac{N}{1} \text{ NaCl.} \tag{4}$$

(5) By actual experiment it was also found

$$1 \text{ vol. of } \tfrac{S}{2} = 1 \text{ vol. of } \tfrac{60}{20}\tfrac{N}{1} \text{ NaCl or 3 vols. of } \tfrac{N}{1} \text{ NaCl.} \tag{5}$$

It is evident from the above that we should have in the serum obtained from a mixture of 1 volume of blood and 2 vols. of $\tfrac{S}{2}$ NaCl an amount of NaCl which is contained in $(6+\tfrac{1}{2}\times\tfrac{3}{25})$ vols. of $\tfrac{N}{1}$ NaCl, *provided no combination has taken place between the corpuscles and NaCl of the* $\tfrac{S}{2}$ *NaCl solution.*

Also 3 volumes of the mixture contain $3\times\tfrac{49}{54}$ or $\tfrac{49}{18}$ volumes of supernatant fluid.

Therefore, $\tfrac{49}{18}$ volumes of the supernatant fluid contain the amount of NaCl which is present in $\tfrac{303}{50}$ volumes of $\tfrac{N}{1}$ NaCl.

Therefore, 1 volume should contain an amount of NaCl that is present in $\tfrac{303}{50}\times\tfrac{18}{49}$, *i.e.*, $2\cdot23$ volumes of $\tfrac{N}{1}$ NaCl. $\quad$ (6)

But by actual experiment it was found that 1 volume of the supernatant fluid contained an amount of NaCl which is present in $1\cdot833$ volumes of $\tfrac{N}{1}$ NaCl.

Therefore, the amount of NaCl contained in $\cdot397$ volume of $\tfrac{N}{1}$ NaCl solution must have been absorbed by the erythrocytes of the blood.

2nd experiment.

Student :

(1) In estimating the volume of the serum compared with that of the blood, the same method as in the former case was adopted.

It was found from this that 1 volume of blood contained $\frac{43}{88}$ or nearly half volume of pure serum.   (1)

(2)   One volume of blood was mixed with 2 volumes of $\frac{S}{2}$ NaCl solution and the mixture was quickly centrifuged. It was found that $\frac{3}{4}$ volumes of the mixture contained $\frac{9}{8}$ volumes of the supernatant fluid.   (2)

Therefore, $\frac{9}{10}$ volume of the supernatant fluid was contained in one volume of the mixture.

(3) By quantitative estimation, the chloride content of this serum was found to be as follows :—

1 volume of serum $= \frac{3}{25}$ vol. of $\frac{N}{1}$ NaCl.   (3)

(4) The quantitative estimation of the chlorides in the supernatant fluid after centrifugalisation of the mixture of 1 part of blood and 2 parts of $\frac{S}{2}$ NaCl solution gave the following result :—

1 volume of the supernatant fluid $= 2$ volumes of $\frac{N}{1}$ NaCl.   (4)

(5) By actual calculation it was found

1 vol. of $\frac{S}{2} = 3$ volumes of $\frac{N}{1}$ NaCl.   (5)

From the above we should have in the supernatant fluid, obtained from a mixture of 2 volumes of $\frac{S}{2}$ NaCl and 1 volume of blood, an amount of NaCl which is contained in $(6 + \frac{1}{2} \times \frac{3}{25})$ volumes of $\frac{N}{1}$ NaCl, *provided that no combination has taken place between the corpuscles and NaCl of the* $\frac{S}{2}$ *solution.*

Also 3 volumes of the mixture contain $3 \times \frac{9}{10}$ or $\frac{27}{10}$ volumes of the supernatant fluid.

Therefore, $\frac{27}{10}$ volumes of the supernatant fluid contain an amount of NaCl which is present in $\frac{303}{50}$ volume of $\frac{N}{1}$ NaCl.

Therefore, 1 volume contains an amount of NaCl which is present in $\frac{303}{50} \times \frac{10}{27}$ or $2\cdot 24$ volumes of $\frac{N}{1}$ NaCl.   (6)

But by actual experiment, 1 volume of the supernatant fluid contained an amount of NaCl which is present in 2 volumes of $\frac{N}{1}$ NaCl solution.

Therefore, the amount of NaCl which is contained in $0\cdot24$ volume of $\frac{N}{1}$ NaCl solution must have been absorbed by the erythrocytes.

3rd experiment.

Student :

(1) Here too in estimating the volume of the serum compared with that of blood, the same method as in the above experiments was adopted.

It was found that one volume of blood contained $\frac{14}{29}$ or nearly $\frac{1}{2}$ vol. of pure serum. (1)

(2) One volume of blood was mixed with 2 vols. of $\frac{S}{2}$ NaCl solution and the mixture was quickly centrifuged.

It was found $\frac{34}{36}$ volume of the supernatant fluid was contained in one volume of the mixture. (2)

(3) By quantitative estimation, the chloride content of the serum was found as follows :—

1 volume of serum $= \frac{11}{100} \frac{N}{1}$ NaCl. (3)

(4) The quantitative estimation of the chlorides in the supernatant fluid after centrifugalisation of the mixture of 1 part of blood and 2 parts of $\frac{S}{2}$ NaCl solution gave the following result :—

1 volume of the supernatant fluid $= 2$ volumes of $\frac{N}{1}$ NaCl. (4)

(5) By actual experiment it was found that 1 volume of $\frac{S}{2} = 3$ of $\frac{N}{1}$ NaCl. (5)

From the above we should have in the supernatant fluid obtained from a mixture of 2 volumes of $\frac{S}{2}$ NaCl and 1 volume of blood, an amount of NaCl which is present in $(6+\frac{1}{2} \times \frac{11}{100})$ volumes of $\frac{N}{1}$ NaCl, *provided that no combination has taken place between the corpuscles and NaCl of the $\frac{S}{2}$ solution.*

Also 3 volumes of the mixture contain $\frac{34}{12}$ volumes of the supernatant fluid.

Therefore, $\frac{3}{2}$ volumes of this fluid contain an amount of NaCl which is present in $\frac{121}{200}$ volumes of $\frac{N}{1}$ NaCl.

Therefore, 1 volume contains an amount of NaCl which is present in $\frac{121}{200} \times \frac{12}{31}$ or $2\cdot34$ volumes of $\frac{N}{1}$ NaCl solution. (6)

But by actual experiment it was found that one volume of the supernatant fluid contained an amount of NaCl which is present in 2 volumes of $\frac{N}{1}$ NaCl.

Therefore, the amount of NaCl which is contained in $0\cdot34$ volume of $\frac{N}{1}$ NaCl solution must have been absorbed by the erythrocytes.

The above method was also adopted to determine whether any combination takes place between the erythrocytes of the rabbit's blood and $\frac{N}{1}$ NaCl, when the former is mixed with two volumes of $\frac{N}{1}$ NaCl solution. The following data were thus obtained : —

(1) Volume of serum $= \frac{51}{100}$ volume of the blood or nearly $\frac{1}{2}$.

(2) By centrifugalisation of a mixture of one part of blood and two parts of $\frac{N}{1}$ NaCl solution, it was found that the volume of the corpuscles was $\frac{2}{3}$ths that of the blood.

Therefore, 2 volumes of $\frac{N}{1}$ NaCl + 1 volume of blood contain $2\frac{3}{5}$ volumes of the diluted serum.

By actual calculation it was found that one volume of serum $= \frac{3}{25} \frac{N}{1}$ NaCl.

Therefore, $2\frac{3}{5}$ volumes of the diluted serum contain an amount of NaCl which is present in 2 volumes of $\frac{N}{1}$ NaCl + 1 vol. of $\frac{51}{100} \times \frac{3}{25} \frac{N}{1}$ NaCl, *provided that no combination has taken place between the corpuscles and NaCl of the $\frac{N}{1}$ NaCl solution.*

That is, $2\frac{3}{5}$ volumes of the diluted serum $= 2\cdot062$ vols. of $\frac{N}{1}$ NaCl or 1 vol. $= \frac{1031}{1313}$ vols. of $\frac{N}{1}$ NaCl $= \cdot7931$ vol. of $\frac{N}{1}$ NaCl.

By actual calculation it was found that

2 vols. of the diluted serum $= 4 \times 4 \times \frac{1}{10}$ vols. of $\frac{N}{1}$ Ag NO$_3$ or one volume $= \cdot8$ vol. of $\frac{N}{1}$ NaCl.

Therefore, the difference between the two calculations is ($\cdot 8$—$\cdot 7931$) vol. of $\frac{N}{1}$ NaCl $=$ $\cdot 0069$ vol. of $\frac{N}{1}$ NaCl, which is within the limits of experimental errors. Therefore, no combination takes place between the erythrocytes of the rabbit's blood and the NaCl of the $\frac{N}{1}$ NaCl and this is also confirmed by the fact that hardly any hæmolysis takes place when one volume of the rabbit's blood is mixed with 2 vols. of $\frac{N}{1}$ NaCl solution, contrary to what is found when one volume of the rabbit's blood is mixed with 2 vols. of $\frac{S}{1}$ or $\frac{S}{2}$ NaCl solution.

These few experiments lead to very important results which have not been observed before. If confirmed, they would definitely prove that when blood is treated with very concentrated NaCl solutions, NaCl enters into combination with the erythrocytes and afterwards destroys their resisting power to hæmolysis. This combination is probably of the nature of *adsorption*.

It is thus seen that my observations agree with those of previous observers in so far that the erythrocytes are generally impermeable to NaCl when treated with certain concentrations of it, but when treated with very concentrated solutions, such as, saturated or half-saturated solution, combination probably does take place and in this latter respect the behaviour of the erythrocytes is different from what takes place when they are treated with comparatively dilute NaCl solutions. When such combination takes place, the erythrocytes are markedly changed in their character and properties, as compared with those that are normal. As a result of this the erythrocytes, for a short time, as pointed out in detail previously, behave like spheres of sponges holding dissolved colouring matter. But, sooner or later, a portion of the outer part of the erythrocytes is separated from its other constituents and as a result of this there is marked hæmolysis as well as complete disintegration of the erythrocytes, as is shewn by the fact that shadow

corpuscles are fewer, when blood is treated with $\frac{s}{1}$ or $\frac{s}{2}$ NaCl solution, than when treated with a hyposmotic NaCl solution. This portion of the erythrocytes is perhaps the cell-globulin of Halliburton and Friend,[1] which was afterwards found to be a nucleo-proteid.[2] It has been stated that the stroma of the erythrocytes contains only a small amount of this substance,[3] but it appears to me to be a very important constituent of the erythrocytes preventing the solution of the erythrocytes in normal blood. It probably exists in combination with the lecithin and cholesterin existing in the walls of the erythrocytes.[4]

[1]   Journal of Physiology, Cambridge and London, 1886, Vol. X.

[2]   Halliburton, Journal of Physiology, Cambridge and London, 1895, Vol. XVIII.

[3]   Schafer, Quain's Anatomy, 1901.

[4]   Schafer's Physiology, Vol. I, 1898.

RESISTANCE OF THE ERYTHROCYTES TO HÆMOLYSIS UNDER CERTAIN ABNORMAL CONDITIONS—INCREASED RESISTANCE OF THE WALLS OF THE ERYTHROCYTES UNDER CERTAIN ABNORMAL CONDITIONS—HÆM-ALKALINITY AND HÆM-SALINITY

I have already observed that if one volume of normal blood is mixed with two volumes of $\frac{N}{20}$ NaCl solution, slight hæmolysis is not infrequently observed, while with $\frac{N}{30}$ it is often distinct or sometimes even well marked. In certain forms of anæmia, 2 volumes of $\frac{N}{20}$ NaCl cause no hæmolysis, while $\frac{N}{30}$ or even $\frac{N}{40}$ NaCl cause very slight or no hæmolysis. In other words the erythrocytes, in some forms of anæmia, resist hæmolysis more than in normal blood. Major McCay, by estimating the hæmosozoic value of serum in certain forms of anæmia, also arrives at the same conclusion and he thinks that this might be due to the presence of an antihæmolysin.[1] This resistance of the erythrocytes to hæmolysis has been already described by me as their specific resistance. In a case of black-water fever this specific resistance was found to be so high as ·585 immediately after the attack was over, while five days after the attack it was so low as ·294. It is perhaps

[1] McCay, Bio-Chemical Journal, Vol. III, 1908.

increased when blood is treated with a mild solution of formol. Thus it was found that when one part of blood is mixed with one part of $\frac{N}{10}$ saline solution and then treated with two parts of $\frac{N}{30}$ saline, the amount of hæmolysis is much greater than when the $\frac{N}{10}$ saline contains 1 per cent formol.

The resistance of the erythrocytes to hæmolysis that is observed in some forms of anæmia led me to investigate if this could be due to any peculiarities in the serum. It was, therefore, found necessary to determine the *hæm-alkalinity* and *hæm-salinity* of these cases and compare them with normal.

Hæm-alkalinity : The method adopted was a modification of that described by Moore and Wilson.[1] In this method not more than 10 c. mm. of serum was required and 50 c. mm. of blood enabled me to estimate both hæm-alkalinity and hæm-salinity. The methods devised by Landois[2], Liebreich[3], Haycraft and Williamson[4], and Kraus[5], for estimating the alkalinity of blood are not, as pointed out by Da Costa,[6] well adapted to routine blood work.

50 c. mm. of blood were taken from the finger, which was sterilized with 5 per cent formol solution, and put into a perfectly dry sterilised tube and then quickly centrifuged. The tube was then closed air-tight to prevent any evaporation and allowed to stand for some hours and again centrifuged, so as to free the serum from all the erythrocytes. 10 c. mm of the serum were treated with a solution of $\frac{N}{100} H_2SO_4$ in a white porcelain shallow capsule, using a

[1] Moore and Wilson. Bio-Chemical Journal, Vol. I, 1906.

[2] Real-Encyclop., 1885, Vol. III.

[3] Berichte d. deutsch, chem. Gessellsch, 1868.

[4] Proceedings, Royal Society, Edinburgh, 1888.

[5] Zeitschr. F. Heilk., 1889.

[6] Da Costa, Clinical Hæmatology.

drop or two of a fresh dilution of an alcoholic solution of di-methyl-amido-azo-benzol in distilled water as an indicator,[1] the first beginning of neutralisation being indicated by a faint rose colour at the side of the solution in the porcelain capsule.

Hæm-salinity :—The salinity of the blood was estimated by treating 10 c. mm of blood with $\frac{N}{100}$ AgNO$_3$ using a solution of K$_2$CrO$_4$ as an indicator.

A series of observations were made on the blood of healthy students as well as of some cases of anæmia and the results obtained are appended in the accompanying tables :—

Salinity and Basic Reactivity of Normal Blood

		Salinity.	Basic Reactivity.	Commencing hæmolysis was brought about by 2 vols. of
1	N. N. A.	·6435%	·200 Normal	$\frac{N}{20}$ NaCl.
2	D. N. R.	·5850%	·180 ,,	Do.
3	R.	·6435%	·160 ,,	Do.
4	S.	·7020%	·160 ,,	Do.
5	H. N. S.	·7020%	·210 ,,	Do.
6	I.	·6435%	·200 ,,	Do.
7	G-	·7020%	·170 ,,	Do.
8	S. C. R.	·7020%	·150 ,,	Do.
	Average	= ·6654%	= ·178$\frac{N}{1}$	Do.

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[1] I generally mix one drop of the alcoholic solution with about 2 c.c. of distilled water just at the time of the experiment. A few drops of this mixture are added to the serum to make it faintly yellow,

Salinity and Basic Reactivity of the Serum in some Forms of Anæmia

		Salinity.	Basic Reactivity.	Red corpuscles and Hæmoglobin.	Disease.	Commencing haemolysis was brought about by 2 vols. of
1	Patu ...	·7605%	·140 Normal	R.B.C. = 1,686,000 Hb=15%	Ankylostomiasis (no œdema)	$\frac{N}{30}$ NaCl
2	Himo ...	·5850%	·095 ,,	R.B.C. = 1,770,000 Hb=13%	Do.	$\frac{N}{40}$ NaCl
3	Panchu ...	·6727%	·140 ,,	R.B.C. = 2,600,000 Hb=60%	...	$\frac{N}{30}$ NaCl
4	... ...	·6724%	·0675 ,,	Hb=5%	Ankylostomiasis (very marked œdema)	$\frac{N}{40}$ NaCl
5	Prayag ...	·6435%	·150 ,,	R.B.C. = 1,920,000 Hb=40%		
6	Bnoirab ...	·8772%	·110 ,,	R.B.C. = 2,180,000	Cancer of the Stomach	$\frac{N}{20}$ NaCl
	Average ...	·7018%	·1170 Normal			

It will be seen from the above that while there was not much appreciable difference in the chlorides of the serum in some of the cases of anæmia compared with the normal, there was rather a distinct diminution in the alkalinity of the serum in almost all of them. This latter fact, so far as I am aware, has not been noted by any previous observer. V. Jaksch, however, by titration of *opaque blood* also finds diminution of alkalinity in all anæmias.[1]

It is evident that the resistance of the erythrocytes to hæmolysis in some cases of anæmia (especially ankylostomiasis) is not due to the presence of any excess of chlorides in the serum. Besides, in some of these cases, I washed the erythrocytes several times with a deci-normal solution of $NaCl^1$ till the supernatant fluid obtained on centrifugalisation was found to be perfectly free from the slightest trace of albumen. One volume of the suspension of the erythrocytes was now treated with 2 vols. of $\frac{N}{30}$ NaCl and it was found that the resulting mixture did not show any hæmolysis at all, while normal blood under similar circumstances showed marked hæmolysis.

The diminution in the alkalinity of the serum in the cases of anæmia cannot, however, explain the increased resistance of the erythrocytes to hæmolysis, because diminished alkalinity increases the tendency to hæmolysis.[2]

The resistance of the erythrocytes to hæmolysis in cases of anæmia is not, therefore, due to anything present in the serum. Whatever may be the cause, it must exist in the red corpuscles themselves.

It may be stated here that there was a distinct increase in the salinity of the serum in the case of cancer of the stomach.

[1]   V. Jaksch, Zeits. F. Klin. Med., Vol. XIII, 1887.
[2]   Schafer's Physiology, Vol. I.

RESISTANCE OF THE ERYTHROCYTES OF BLOOD THAT HAS BEEN KEPT SHED FOR SOME TIME TO HÆMOLYSIS—SPONTANEOUS HÆMOLYSIS—EFFECT OF $\frac{N}{10}$NaCl UPON SPONTANEOUS HÆMOLYSIS—HÆMOLYSIS AND COAGULATION—EFFECT OF X-RAYS UPON THE RESISTANCE OF THE ERYTHROCYTES TO HÆMOLYSIS

If blood is kept in a perfectly sterilized tube, evaporation being prevented by carefully sealing the mouth of the tube, we find that the resistance of the erythrocytes to hæmolysis diminishes in course of time.

Thus we had in some cases :—

1. Freshly drawn blood+2 vols. of $\frac{N}{20}$NaCl = faint hæmolysis.

2. Blood kept shed for 24 hours + do. = do.

3. Blood kept shed for 48 hours + do. = slight hæmolysis.

4. Blood kept shed for 80 hours + do. = distinct hæmolysis.

From the above, it will be seen that the erythrocytes began to lose their power of resistance to hæmolysis eighty hours after the blood was shed and kept at the temperature of the room which was 29°C. This loss of the resisting

power must be explained as a sign of the commencement of the loss of vitality in the erythrocytes. Similarly it has been shown that mammalian blood kept in the cold in a flask exposed to the influence of ice water, may retain in functional activity for 4 or 5 days, and removed from the body for a longer period of time and then returned to the circulation, the red corpuscles rapidly undergo destruction.[1] In my observations the power of resistance to hæmolysis was distinctly lost eighty hours after the blood was shed. It will be seen that my method of treating blood with $\frac{N}{20}$ NaCl solution and observing the amount of hæmolysis that has taken place in the mixture, is a much simpler way of determining when the erythrocytes are beginning to lose their vital activities than the difficult and complicated method of returning the erythrocytes to the circulation and then watching whether they are undergoing destruction or not.

The erythrocytes undergo spontaneous hæmolysis in blood that has been kept shed for sometime, and this is independent, as has been pointed out by Stewart,[2] of any bacterial infection. I have observed in some of my experiments no spontaneous hæmolysis taking place in the blood even eighty hours after the blood was kept shed in a perfectly sterilised and closed tube at the temperature of 20°C. In other cases I found spontaneous hæmolysis starting earlier. The circumstances which enhance or retard this have still to be investigated. One fact that I have noticed in some of my cases is the retardation of spontaneous hæmolysis by the removal of the clot that was formed in the blood and also by the addition of a little sterilised $\frac{N}{10}$ NaCl solution. These conclusions have, however, to be tested by more extended observations. If corroborated, they might lead to the conclusion that pro-

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[1] Landois, Text-Book of Human Physiology.

[2] Stewart, Journal of Physiology, Vol. XXIV, 1899.

bably something of the nature of a hæmolysin is produced by the disintegration of the leucocytes that are entangled in the clot.

Effect of X-rays on the resistance of the erythrocytes to hæmolysis :—

It has been observed that electric shocks passed through blood, if sufficiently strong, cause hæmolysis. The effect of X-rays is, however, different. I have found that blood exposed to X-rays for 20 to 30 minutes does not show less resistance to hæmolysis than the blood that has not been thus acted upon. Thus I have seen in some of my cases :—

1. Blood, not exposed to X-rays $+2$ vols. of $\frac{N}{20}$ NaCl $=$ slight hæmolysis.

2. Blood exposed for 20 minutes to X-rays $+ 2$ vols. of $\frac{N}{20}$ NaCl $=$ slight hæmolysis.

CONSTITUTION OF THE ERYTHROCYIES REVEALED FROM THE PHENOMENON OF HÆMOLYSIS. CHEMICAL COMBINATION AND MASS ACTION. MECHANISM OF CRENATION. ENTRANCE OF WATER INTO THE ERYTHROCYTES AND THEIR RESISTANCE TO HÆMOLYSIS PARTLY A VITAL PHENOMENON

Under what conditions does hæmoglobin exist•in red corpuscles ?

We have already pointed out in the last chapter that hæmoglobin, as it exists inside red corpuscles, is in the form of a suspension.

Further investigations carried on by me lead to the conclusion that there exists a union allied to chemical combination between the hæmoglobin of the erythrocytes and other portions of their structure. If we examine the resistant corpuscles under the microscope it is easily seen that a large number of them have undergone marked changes in shape, size, and in the amount of contained hæmoglobin. The appearances very much resemble what we observe in cases of anæmia. Some of the corpuscles are resistant in the sense that they have not at all discharged their hæmoglobin. But there are others which show marked diminution in the amount of continued hæmoglobin. Some

show changes in the distribution of contained hæmoglobin as compared with the normal. Evidently there has been a partial escape of hæmoglobin from these corpuscles. The question may be asked what it is that prevents the remaining portion of the hæmoglobin from being completely discharged? The most probable assumption is that the process of hæmolysis by hypotonic saline or distilled water is to some extent allied to *mass action* that takes place in chemical reactions. In other words, there probably exists a union allied to chemical combination between the hæmoglobin of erythrocytes and other portions of their structure. *Hæmoglobin, therefore, exists inside erythrocytes in the form of a suspension in combination with some portions of the substance of erythrocytes.*

Another explanation may be offered of the above phenomenon. Let us now consider the structure of the partially hæmolysed erythrocytes. It can be shown that on addition of $\frac{N}{10}$ NaCl solution to a sample of blood hæmolysed by distilled water, a turbidity again appears which must be due to the shrinking of the corpuscles. It thus appears that the osmotic capabilities of the cellular framework of these cells have not been altogether destroyed. To consider, therefore, that the erythrocytes are simple bags of membrane containing hæmoglobin and rupturing when treated with distilled water, seems to be untenable. Such simple bags of membrane when once ruptured will allow water to enter into their structure, when washed with $\frac{N}{10}$ NaCl, till the whole of the hæmoglobin contained inside them is completely washed out. Therefore, the fact that such partially hæmolysed corpuscles contain, when washed with $\frac{N}{10}$ NaCl, some amount of hæmoglobin inside their structure renders the simple bag-theory untenable. On the other hand, we may consider that they consist of specially constructed bags with membranous partitions containing hæmoglobin, as represented in the diagram. Some
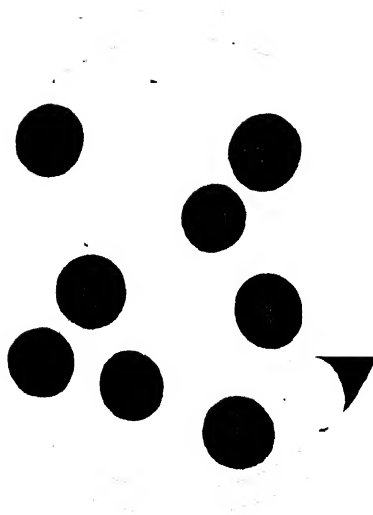
Diagrammatic representation of partitioned walls inside red corpuscles

of the membranes rupture in the partially hæmolysed corpuscles, while others remain unruptured.

We have already observed that if a suspension of erythrocytes in $\frac{N}{10}$ NaCl solution, is placed over a slide and then allowed to dry gently, we find that the dried corpuscles when treated with $\frac{N}{10}$ NaCl solution, dissolve completely. Let us consider what happens when the erythrocytes are drying on the slide. They tend to stick to one another and to the slide. On the other hand, as evaporation goes on, they tend to contract. As a result of these two antagonistic processes there is perhaps rupture of their membraneous structure, and they dissolve when they come in contact with what would be an isotonic solution in the case of undried erythrocytes. Their complete solution indicates complete disruption of their cellular framework in the process of drying up.

This complete disruption is either purely mechanical due to the rupture of all the membraneous portions inside the erythrocytes or the removal of H_2O during evaporation brings about a complete change in the chemical constitution of the erythrocytes converting them into particles soluble in saline of any strength, just as a lump of sugar dissolves in water. This latter view best explains the complete solution of erythrocytes when treated with saturated solution of NaCl in water. In the process of desiccation of the erythrocytes by the saturated NaCl solution water is removed from them. A portion of this water is perhaps in chemical combination with the erythrocytes and this compound is broken up when they are allowed to dry on a slide or are treated with saturated NaCl solution.

Another explanation may be offered to explain why erythrocytes that are drying on a slide dissolve in $\frac{N}{10}$ NaCl solution. When a suspension of the erythrocytes in $\frac{N}{10}$ NaCl solution is dried over a slide, the NaCl solution tends to become more and more concentrated and consequently

a stage comes at which it becomes saturated and hence the erythrocytes come to be in contact with a saturated NaCl solution and then they behave like what have been already described as salted erythrocytes and therefore dissolve when further treated with $\frac{N}{10}$ NaCl solution.

We thus see

1. (Blood + saturated NaCl solution) + $\frac{N}{10}$ NaCl solution = laking.

2. Blood allowed to dry on a slide + $\frac{N}{10}$ NaCl solution = laking.

From these facts we may conclude that hæmoglobin as it exists inside erythrocytes probably exists as a compound with H_2O and that this compound is decomposed by abstraction of water during evaporation or by treatment with very concentrated NaCl solution ($\frac{S}{1}$ or $\frac{S}{2}$).

The above explanation is open to the objection that when the erythrocytes are treated with moderately strong NaCl solutions we find crenation of the corpuscles taking place without hæmolysis.

It is, however, possible that the portion of the H_2O, which is not in chemical combination with hæmoglobin, comes out of the erythrocytes when the latter are treated with moderately strong NaCl solution giving rise to crenation of the corpuscles, while the portion, that is in chemical combination, comes out when the erythrocytes are treated with saturated NaCl solution giving rise to their complete hæmolysis. This compound may be represented as H—X—OH.

The holding of hæmoglobin within the erythrocytes is, to some extent, a *vital phenomenon*. This is partly proved by the phenomenon of spontaneous hæmolysis and partly also by the fact that one volume of freshly drawn blood mixed with 2 vols. of $\frac{N}{10}$ NaCl solution shows only a faint hæmolysis, the same shows a distinct hæmolysis when treated in the same way after it has been kept shed for 60

to 80 hours.   This inadequacy of the erythrocytes in blood
that has been kept shed for some time, to hold hæmoglobin
within their substance is independent of any bacterial
infection and is probably a sign. of commencing loss of
vitality.   This loss of vitality takes place much more quickly
when the erythrocytes dry up than when they are kept in
a closed tube, evaporation being thereby prevented.

We thus conclude that *hæmoglobin exists inside erythro-*
cytes in suspension and in combination with H₂O.   Its
existence inside erythrocytes is partly a *vital phenomenon.*
In this connection we must also refer to the observations
of Moore, who has shown that the ions inside red corpuscles
are attached to the hæmoglobin, the phosphates being more
firmly held than the chlorides.[1]   Similarly, the experiments
of Stewart lead to the conclusion that hæmoglobin as it
exists inside red corpuscles, is united with the stroma, while a
portion of the electrolytes remain in solution as such, another
portion being combined with the stroma.[2]   •

The hæmolysis of erythrocytes by hyposmotic or hyper-
    osmotic NaCl solutions :—

We just now stated that hæmoglobin exists inside
corpuscles in combination with H₂O and also with the phos-
phates and the chlorides.   If we assume that this compound
of hæmoglobin is decomposed by excess of water or by a
very hypertonic NaCl solution or during evaporation, then
the phenomenon of hæmolysis can be explained without
assuming that the walls of the erythrocytes are ruptured
during hæmolysis.   When water enters the erythrocytes in
excess, this compound of the hæmoglobin is decomposed
and dissolves in the excess of water and this dissolved
hæmoglobin comes out of the erythrocytes by a process

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[1] Further Advances in Physiology.   Edited by Leonard Hill. Edward Arnold,
1909.

[2] Journal of Physiology, Vol. XXIV.

allied to filtration or as Stewart suggests, even by diffusion,[1] though hæmoglobin is non-dialysable through ordinary membranes. If the water entering the erythrocytes is not in excess, then there is only distension of the erythrocytes but not decomposition of the compound of hæmoglobin. In other words, this compound is decomposed only in excess of water. Similarly when the erythrocytes are treated with saturated or half-saturated NaCl solution, then also the same compound is decomposed and the erythrocytes behave like spheres of sponges containing dissolved colouring matter and hæmoglobin comes out of them as through a filter. *In other words, this compound is decomposed by excess of water as well as by excessive abstraction of water.*

The amount of hæmoglobin that will come out of an individual erythrocyte during the process of laking by hyposmotic NaCl solution will depend upon the hypothetical compound of hæmoglobin that is decomposed and this depends upon the masses of the interacting compounds, namely the compound of hæmoglobin and the water that enters the erythrocytes. This latter depends greatly upon *osmosis* and to some extent upon the permeability of the outer portion of the erythrocytes to water.

This permeability, as has been already pointed out, is not always the same. The behaviour of crenated corpuscles towards $\frac{N}{10}$ NaCl solution and the presence of crenated corpuscles in the blood of some forms of anæmia, prove that the permeability of erythrocytes to water is variable. Crenation probably means commencing loss of vitality of the corpuscles and is associated with diminution of their permeability to water. This diminution evidently takes place in spite of the force of *osmosis*. Crenation, therefore, is not simply due to ex-osmosis of water from the corpuscles, as is generally supposed to be the case. If the latter were

the only cause of crenation, then one would expect to observe the greatest amount of crenation when blood is treated with saturated NaCl solution, but I have shown that this is not the case.

We have, however, already pointed out that, under normal circumstances, this permeability is the same in all the corpuscles. But in abnormal corfditions, e.g., during crenation and also perhaps in disease, this permeability of the corpuscles is not the same as that of the normal ones.

It is a law in physical chemistry that osmosis is independent of the membranes through which it takes place. As the permeability of corpuscles varies under different conditions, it is doubtful whether the law holds good absolutely in their case. It is on the other hand, likely that they possess a *specific permeability* which is the same in normal conditions but may vary under abnormal conditions. This *specific permeability* should be a subject for further investigations. Apart from osmosis, the amount of water that would enter the erythrocytes, is determined by the activity of the outer portion of the erythrocytes, which constitutes their specific permeability and which is probably dependent upon their *vitality*. Similarly, resistance to hæmolysis is to some extent, dependent upon the same cause. Permeability of erythrocytes to water, is to some extent, comparable to the absorption of oxygen into the lung tissue which is partly due to epithelial activity, as has been shown by the experiments of Bohr and Haldane. The existence of the specific permeability is also proved by the experiments of Moore and Roaf[1] showing that the freezing points of the corpuscles and the serum are not exactly the same and this could not be the case if there were a perfect osmotic equilibrium between the corpuscles and the serum as one would expect to find if the walls of the erythrocytes behaved like ordinary membranes.

[1] Bio-Chemical Journal, 1908.

A CASE OF SEPTIC ENDOCARDITIS

Bhubun, æt. 26, Hindu female, was admitted on the 14th September, 1899, for the treatment of an irregular type of fever of nearly a fortnight's duration. There was history of a vaginal discharge for about three weeks and painful swelling of the knees and ankles for about a week.

Condition on admission.—Patient looked very ill; face was anxious; skin was hot and dry; tongue was coated, dry and tremulous; marked *subsultus tendinum* was present; the bigger joints of the body were tender and painful, especially the right knee which was hot, red and somewhat tense. The liver and spleen were slightly enlarged. The heart sounds were regular and somewhat sharp; no bruit or rub was audible over the prœcordia; cardiac dulness was normal. There was nothing abnormal in the breath sounds.

Subsequent history.—The patient was put on large doses of salicylate of soda for two days without the slightest benefit resulting. On the sixth day the swelling in the joints, especially the right knee, diminished to a remarkable extent, but the patient began to suffer from low muttering delirium. On the seventh day acute bed sores appeared over the sacrum and inferior angles of the scapulæ and the skin had an icteroid tinge. On the tenth day a few vesicles appeared over the hands and ears which soon became pustules. The heart sounds were normal throughout and the cardiac dulness was not increased; the pulse became irregular at the last stage. The patient died on the twelfth day after admission.
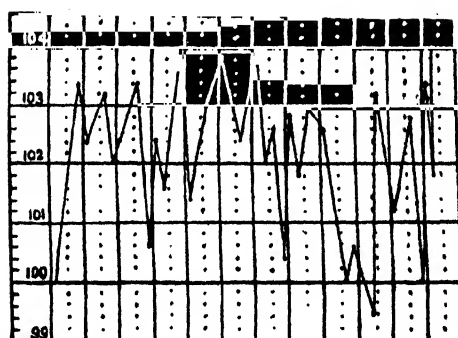
A case of septic endocarditis

On *post-mortem* examination the pericardiu m was found to contain an ounce of turbid sero-purulent fluid; between the anterior and middle cusps of the aortic valves, there was a small nodule about the size of a pea extending to half of the anterior cusp. On piercing the vegetation a hole was made in the valve and it was removed very easily ; a condition similar to that of the aortic valves was found in the posterior cusp of the mitral valve; there was no vegetation on the tricuspid valve.

The uterus was healthy, but there was some purulent discharge from the vaginal walls, and some adherent blood tinged mucus was found in the body of the uterus.

In both the knee joints, the synovial membrane was vascular, especially on its free edge, and a small quantity of purulent fluid was found within these joints. There was no erosion of cartilages.

Remarks.—This case was an anomalous one, at any rate it was so during life. The interest of the case lay clinically in the fact that the local signs did not seem commensurate with the general state of the patient. Throughout the course of the case the only points upon which the diagnosis had to be based were the presence of pronounced articular inflammation and a remittent type of fever which seemed to comport itself along no definite course. Although carefully sought for repeatedly, no definite signs of cardiac change could be discovered. The area of cardiac dulness remained unaltered; the sounds were as normal as any patient suffering from prolonged remittent fever could reasonably be expected to have. There was no evidence at any time of malignant endocarditis. Such cases with such a lack of definite proportion of symptoms to physical signs actually fall within the category of that class of cases which is described as pyæmic. It could not be said that there were any signs of multiple pyæmic suppurations, although they were carefully sought for, except the articular inflammation.

A provisional diagnosis of malignant endocarditis was made, for it seemed to be the only condition left which could explain such an anomalous series of symptoms. Day after day the heart was carefully examined for the evidence of any abnormal signs but none were ever found. The *post-mortem* came as a surprise; it was a case of pyæmia so far as the joints were concerned, but it was also a case of malignant endocarditis of which there was abundant evidence *post-mortem*. The case is probably best regarded as infective endocarditis of the pyæmic type; this will explain both the endocardial as well as the arthritic lesions. But the absence of arterial pyæmia of Wilks which forms one of the salient features of malignant endocarditis, except the articular lesions which might be supposed to be embolic, and of any suppurative phlebitis and visceral metastatic abscesses which are almost constantly present in pyæmia, is difficult to explain. The source of infection was undoubtedly from the vagina being probably gonorrhœal; the gonococcus was not, however, demonstrated in the vaginal discharge or the vegetations. The presence of malignant endocarditis due to the gonococcus is certainly possible, and has been pointed out in a recent paper in the *British Medical Journal* to have been long recognised in the Guy's Hospital, and to have also been demonstrated by Thayer and others. Such cases of genuine gonorrhœal endocarditis are, however, characterized by marked endocardial symptoms as mentioned in *Allbutt's System of Medicine*. On the other hand, the case might be due to a secondary septic infection in the course of gonorrhœa; this will explain why the case had more of the symptoms of malignant endocarditis of the septic or pyæmic type than of the cardiac type.
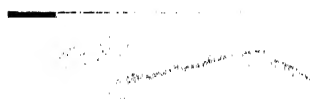
A case of post-hemiplegic athetosis

A CASE OF POST-HEMIPLEGIC ATHETOSIS

The patient named Khetter, æt. 25, a Hindu male, belonging to the railway mail-service, stated that on 17th July, 1899, twelve hours after a fatiguing swim, he began to suffer from giddiness and weakness in the left side of his body. Two or three days later he was admitted into Lt.-Col. Harris's ward for treatment of hemiplegia with the following symptoms :—

(1) Left facial palsy.

(2) Complete motor paralysis of the left upper and lower extremities without any sensory disturbance.

(3) Exaggeration of the knee-jerk on the left side.

There was no history of syphilis. There was history of occasional transient fits of unconsciousness for about a year. The patient left hospital having almost recovered. One night about a month after leaving hospital, he was awakened from sleep by an involuntary flexion of the left middle finger which, since then, has more or less persisted in a series of mobile spasmodic movements. This condition gradually increased and affected the other fingers, interfering very materially with his work, and it was on this account that he again sought relief on 13th December, 1899. The symptoms on admission were :

(1) Slight disorder of sensation in the fingers of the left hand. The following was the table of tactile sensibility of

both the upper extremities as measured with the æsthesio-
meter.

Tactile sensibility	Right side	Left side
(a) Dorsum of 1st phalanges of fingers	$\frac{7}{12}$ in.	2 in in middle finger. $1\frac{3}{4}$ in. in index finger. 1 in. in the thumb.
(b) Palm of hand	$\frac{5}{12}$ in.	$1\frac{1}{2}$ in.
(c) Back of hand	$1\frac{1}{8}$ in.	2 in.
(d) Forearm	$1\frac{1}{2}$ in.	$1\frac{3}{4}$ in.
(e) Dorsum of foot	$1\frac{1}{2}$ in.	$1\frac{1}{2}$ in.

(2) Strength of the grip of the hands as measured with
the dynamometer :

    (a) Left hand   ...   .   20 ℔s.

    (b) Right hand   ...   .   70 ℔s.

    (3) Marked increase of the knee-jerk on the left side.

    (4) Athetoid movements in the fingers of the left hand.
There was a slow mobile spasm affecting mostly the
interossei and the lumbricales of the left hand. The amount
of spasm in these muscles varied from time to time and thus
slow, successive, inco-ordinate movements of flexion, extension,
pronation and supination of the fingers were produced. The
movements could be compared to those of tentacles of the
cuttlefish (Gowers). During the execution of some of the
movements the interphalangeal joints passed into a state of
subluxation. Sometimes the distal phalanges were extended,
showing that the long extensor also partook of the spasm.
Sometimes the 1st phalangeal joint of the middle finger was
strongly flexed with the thumb closely pressing against it,
and sometimes there was flexion of the last phalanges of one
or more fingers, showing that the long flexors of the fingers
also partook of the spasm. This implication of the long

flexors was noteworthy which, as Gowers says, never takes place. Rarely the fingers assumed a position which is compared to that of the contracture of the type of extension in chronic articular rheumatism (Charcot). Occasionally, there was a simulation of some of the above movements by the fingers of the right hand, though they were hardly so marked as to justify the case being regarded as one of double athetosis. Two of the positions assumed by the fingers are given in the accompanying photographs. Rarely, there was an involuntary hyperextension of the left big toe especially during walking. The movements disappeared during sleep and could be controlled and limited only for a short time by position and extraordinary effort of the will. The phenomena, as pointed out by Von Zeimssen, seemed to have partially the character of associated movements, for while the fingers moved the arm became rigid and hard. The spasm disappeared when the wrist or the fingers were rigidly flexed, but after a time it started again, somtimes slowly and sometimes suddenly. The spasm markedly interfered with a voluntary act, so that the patient had to wait till it passed off. There was not the slightest contracture in any of the fingers.

(5) Besides the above there were the negative symptoms of absence of motor paralysis of any cranial or spinal nerves and of wasting of any groups of muscles.

Remarks.—The diagnosis of a case of athetosis as a post-hemiplegic state is simple when one is alive to the character and significance of its symptoms. It might possibly be thought to be due to disseminated sclerosis, paralysis agitans, to paramyoclonus multiplex when it attacks the muscles of the arm and face which it very rarely does after hemiplegia, to convulsive tic, chorea spastica, tetany and localized chorea of Sydenham; in none of these conditions, however, are the movements truly athetoid. The possibility of hysterical simulation of the movements should be borne in mind. The

purposeless nature of the movements and the absence of hysterical stigmata should determine the diagnosis. The condition, as Drewry says, is also impossible to be imitated even by the most skilful malingerer. Some authors, including, among others, Hammond and Gray, seem to make an unfortunate confusion by including post-hemiplegic athetosis under post-hemiplegic chorea ; but the latter, properly speaking, refers to chorea-like movements sometimes occurring in the affected limbs after hemiplegia, and should have nothing to do with the slow and peculiarly characteristic movements of athetosis.

The seat and nature of the lesion in athetosis have not yet been satisfactorily worked out. Hammond, who first described the condition, supposed the seat of the affection to be in the intracranial ganglia or in the upper regions of the spinal ganglia. He also states that one probable seat of lesion is the corpus striatum. Von Zeimssen supposed it to be due to changes, partially circumscribed, in the centres of motor innervation. Hitzig supposed it to be due to irritation of these centres. Beevor considers it to be due to lesions in the motor regions of the cortex, and in favour of this view might be cited his case published in the *British Medical Journal*, in 1890. Osler, however, says that in cases of athetosis occurring in adults, the lesion is not in the cortex. Gowers says that a distinction must be made between the cases occurring in adults and those in childhood. He thinks that in the former the disease is situated in or outside the optic thalamus, or in some cases in the posterior part of the internal capsule. In Gould's Year book of Medicine and Surgery for 1899, it is stated that Von Kunn collected fourteen cases from literature, in seven of which there was localized disease of the corpus striatum, in four of the optic thalamus, in two of the porus and in one a softening involving the optic thalamus and corpus striatum.

As regards the nature of the lesion it has been supposed to follow mostly cerebral softening causing extensive slight damage. James Taylor in his article on cerebral palsies of children states that cases of paralysis, followed by athetosis in adults are mostly the results of accident. In the present case no history of accident could be discovered. The history of hemiplegia following over-exertion might be suggestive of embolism or hæmorrhage, in which case, however, the paralysis would more immediately follow the over-exertion ; nor were there any signs of heart lesion which might be suggestive of a possible source of emboli. The excited action of the heart and the increase in blood pressure consequent upon over-exertion in swimming might have led to rupture of some diseased vessels in the motor tract of the brain. The diseased condition of the vessels might have been the result of syphilis of which the patient, however, denied any history. Besides, extreme headache which forms one of the characteristics of syphilitic disease of the vessels of the brain was absent in the present case. It might be that the association of the over-exertion with the hemiplegia was an accidental one, and that the occasional transient fits of unconsciousness might represent the minor form of epilepsy (*petit mal*), with which the athetosis was connected, as many of the recorded cases have been.

The present case was of interest as it was a case of athetosis in an adult unassociated with hemianæsthesia and vasomotor change, and following an attack of marked hemiplegia after it was completely recovered from. Another point of interest in the present case was the fact that the long flexor and extensor muscles of the fingers partook of the spasm to some extent, which Drewry says are rarely affected ; while, Gowers says, as already mentioned, the long flexors are never affected.

References

Gower's Diseases of the Nervous System.

Charcot's LeCon de Mardi, 1887.

Charcot's Diseases of the Nervous system (New Sydenham Society, 1888).

Zeimssen's Cyclopædia of the Practice of Medicine.

Hammond's Diseases of the Nervous System (1873).

Beevor's Diseases of the Nervous System.

Gould and Pyle's Anomalies and Curiosities of Medicine.

Osler's Medicine.

Allbutt's System of Medicine, Vol VII.

NOTE.—The present-day views about the pathogenesis of athetosis are given below from Brain and Strawart' *Recent Advances in Neurology* (1929) :—

Owing to the similarity between athetosis and chorea, all authorities seem to be agreed that the pathogenesis of the two conditions is very similar. Most continental authorities emphasise the striatal origin of these disturbances. Forester may be taken as an exponent of this view. He regards the globus pallidus as a centre concerned with certain complex movements of reaction, expression and so on. He considers that the caudate nucleus and putamen act as regulators and inhibitors of pallidal function. Widespread disease of the striatum, therefore, results in the escape of the pallidum from its normal inhibitions, and the result is athetosis, a riot of uncontrolled expressions and movements of considerable complexity. In explanation of chorea, Forester draws attention to the observation of Vogts that there exist two types of cell in the striatum—large and small. He considers that the function of the small cells may be co-ordinative and that of the large cells inhibitory of the activity of the pallidum. In Chorea he postulates a lesion affecting the small cells only, so that there results only an ataxia of the pallidal functions and not their escape from inhibition. Vogts and Jacob hold very similar views. Ramsay Hunt believes that chorea results from a lesion of the small cells of the corpus striatum, while outfall of the large cells produces Parkinsonian rigidity. He regards athetosis as a mixture of chorea and rigidity, resulting from injury to both types of cell.

Kinnier Wilson is unable to accept a striatal origin for chorea and athetosis. He stresses the complex nature of the involuntary movements in chorea and also the fact that they are abolished on the affected side by a complete hemiplegia. He regards chorea, therefore, as a disorder of cortical function. Taking into consideration the situation of lesions known to give rise to chorea, he assumes the existence of an afferent path to the motor cortex from the cerebellum by way of the superior peduncle through the hypothalamic region to the thalamus and so to the cortex. The result of a lesion of this path is a disorder of voluntary movement, together with involuntary movements produced by " a persistent (or intermittent) stream of disordered cerebello-cerebral afferent stimuli "

According to another conception, chorea is a manifestation of uncontrolled activity of midbrain centres. Thus Jacob, although he recognises chorea of striatal origin, also believes that the chorea-athetoid phenomena which appear in connection with lesions in the neighbourhood of the red nucleus, hypothalamus and superior cerebellar peduncle are to be attributed to the release of the red nucleus and brain-stem from cerebellar or pallidal control. A similar conception would seem to be implied in Martin's view that chorea is produced by a lesion of the corpus Luysi. Editor.
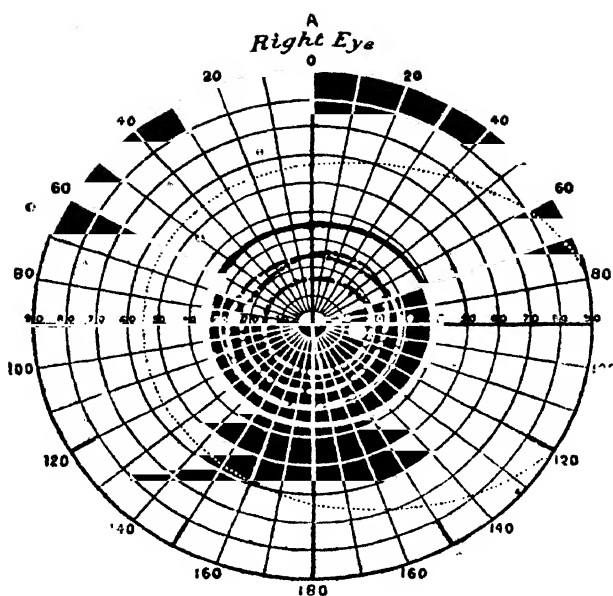
Field of vision in a case of hysteria
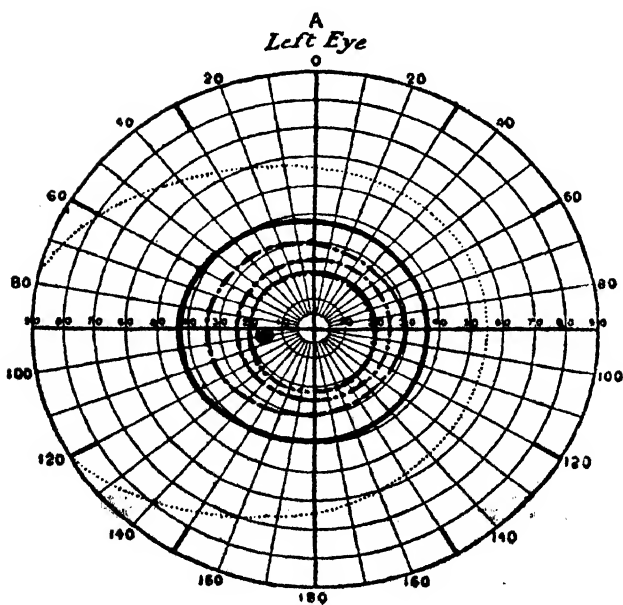
Field of vision in a case of hysteria

FIELD OF VISION IN A CASE OF
HYSTERIA

Patient, named Miss R., æt. 22, was admitted on 19th April, 1900, into Lieutenant-Colonel Bomford's wards in the Medical College Hospitals, Calcutta, for the treatment of sleeplessness, anorexia and pain in front of the chest, all of which came on after her brother's illness. Shortly after admission into hospital she began to suffer from inability to protrude the tongue, clavus hystericus, aphonia, cramps in the toes and fingers, and tenderness in the left iliac region. She had no fits in hospital. Examination for sensation showed bilateral anæsthesia over the face, forearms, abdomen and the legs. The ophthalmoscopic examination of the eyes presented nothing abnormal in either fundus. The visual fields taken on 23rd April, 1900, are represented in the accompanying diagrams.

It will be seen that the visual fields presented the following characters :—

(1) Concentric narrowing of the field of vision in both the eyes.

(2) The boundary of the red was more towards the periphery than that of the blue in the visual field of the left eye.

(3) Crossing of the boundaries of the red and blue in the visual field of the right eye though in most of the meridians the blue was inside the red.

(4) Central vision was intact in both the eyes.

The characteristic feature of the visual field in hysteria is its concentric contraction, with more or less reversion or transposition of the colour fields. This condition of concentric contraction of the visual field was described by Von Graefe, though not quite correctly, as anæsthesia retinæ. In hysteria the boundaries for the different colours are often abnormal, red having a wider boundary than blue, or the boundaries for red and blue cross each other. Sometimes the zone existing between the outer limit of the field for white and that for colours may, according to Buzzard and Head, be obliterated so that they are visible within the same limits as white. Sometimes, as has been pointed out by Parinaud in one of his diagrams, red has even a wider boundary than white. On the other hand, it may be impossible to examine the colour-fields at all in hysteria, all colours being styled dark or black. Sometimes again peripheral vision is entirely abolished, perception being limited only to central vision.

Forester has shown that if the fixation point is moved from the centre to the periphery, the field in each of the meridians is larger than when the object is moved from the periphery to the centre. The visual field in hysteria may diminish in size during examination, either through fatigue, or effort at fixation, and also by a convulsive seizure, just previous to examination which may even lead to a temporary amaurosis. Parinaud has shown that by puncturing the skin, the extent of the visual field is increased, until in certain cases it is rendered normal. Sometimes, again, when the amblyopia is unilateral, cutaneous excitation produces a transfer, the narrowing of the visual field disappearing in the affected eye and developing in the healthy one. Dyschromatopsia may be present in hysteria. It is quite characteristic when present—green and blue are the colours that disappear first and red disappears last.

Hysteria may also require to be diagnosed from insular sclerosis, and here too the visual field may be of some help. Regular concentric contraction of the visual field is very rarely found in insular sclerosis. Uhthoof quoted by Marie describes the following changes in the visual field in insular sclerosis :—

(1) Central scotoma without change in the periphery of the visual field.

(2) Central scotoma associated with contraction of the periphery of the visual field.

(3) Irregular peripheral contraction of the visual field, central vision being normal.

(4) Very rarely concentric contraction of the visual field, the type resembling that of hysteria.

In insular sclerosis the zone between the outer limit of the field for white and the outer limit of the field for colour is maintained, unlike that of hysteria. Dyschromatopsia may also be present in insular sclerosis, but it resembles more the tabetic than the hysterical variety.

The contraction of the visual field in optic atrophy consists in a regular contraction, or in the form of a sector. Sometimes one-half of the field is lost. Sometimes again there is an irregular scotoma in the middle of the field. There is frequently dyschromatopsia—green, red, yellow and finally blue, disappearing successively.

The diagnosis of the hysterical field of vision from that of neurasthenia is often difficult to make. According to Lœwenfield concentric narrowing of the visual field, which is so frequent in hysteria, is not observed in typical neurasthenia, but this fact is not admitted by others. Thus Swanzy describes concentric contraction being often present in neurasthenia. In neurasthenia there is often rapid exhaustion of the nervous visual apparatus during examination, so that there are frequent changes in the size of the visual field. This might also be present in hysteria. Sometimes, in

neurasthenia the field of vision narrows in a spiral direction during examination.

Hysterical amblyopia is generally easily diagnosed from that of the toxic type. In alcoholic amblyopia, there is defective central vision, as well as a central scotoma for red and green. Sometimes colour defects occur in the peripheral visual field, and, very rarely, there is simply contraction of the colour-fields without central scotoma. In tobacco amblyopia the peripheral boundaries of the visual fields are normal, and in the centre of each visual field there is a colour scotoma for red and green, usually oval and stretching from fixing point to the blind spot, the pointed end being towards the blind spot. In severe cases there is scotoma for blue and yellow, and sometimes there are small absolute defects, generally at the nuclear spot. In the dyschromatopsia of both of these conditions, the scotoma develops from the centre extending towards the periphery. Sometimes, however, in hysteria there may be all the symptoms of toxic amblyopia.

The diagnosis of the hysterical field of vision from that of traumatic neurosis is often difficult to make. According to Oppenheim, unilateral or bilateral contraction of the visual field is its most important symptom. However, the relative position of the colour boundaries is seldom altered as is often the case in hysteria.

The field of vision in epilepsy resembles that of hysteria, but in it there is no transposition or reversion of the colour. Besides the contraction, as Charcot points out, is only temporary, though Oliver states it might sometimes exist as a chronic condition.

References

System of Diseases of the Eye. By Norris and Oliver, Vol. IV.
• Marie's Diseases of the Spinal Cord.
Juler's Ophthalmic Science and Practice.
Swanzy's Diseases of the Eye.
Knies on the Eye in General Diseases.
Journal of the American Medical Association, Sept. 26, 1891

Note :   The so-called characteristic features of the visual fields in hysteria which were thought at one time to be of great diagnostic value are now considered to be practically of no importance.   It has been definitely decided that field changes which are functional are caused not by organic lesions of the visual path.   The interference is with the will of the patient and such functions as perception, appreciation, attention and response are involved, in other words, it is the patient who is at fault and not his or her visual mechanism.   These field changes present evidences of more complete suggestion as Roenne, Hurst and Symns and others have pointed out that there is no characteristic perimetric sign, the changes resulting from the patient's imagination together with the method of examination.   They distinctly pointed out that it is a product of the perimeter and that if some less suggestive means be employed to examine the fields no contraction will be found. —Editor.

A CASE OF ACUTE YELLOW ATROPHY OF THE LIVER

The following case came under my observation in my ward in the Mitford Hospital, Dacca, and is published on account of the extreme rarity of the disease :—

The patient, named Ahir, Hindu, male, æt. about 36 years, was admitted on the 19th June, 1901, into the Mitford Hospital, Dacca, for the treatment of jaundice from which he had been suffering for about ten days, with history of constipation and fever for about a week ; no history of syphilis ; no history of chill or mental disturbance preceding the attack of jaundice.

Condition on admission.—Extremely jaundiced, corneal ulcer present in both the eyes. Tongue, dry and brown ; sordes on the lips and teeth ; apathetic and stupid. The liver dulness extended from the sixth interspace to just half an inch below the costal arch in the right nipple line, and there was slight tenderness over the hepatic and epigastric regions.

The diagnosis of the case on admission was one of acute catarrh of the duodenum.

Subsequent history of the case.—The patient became more and more dull and apathetic. The bowels were extremely constipated and were moved by purgatives and enemata, and the stools were pale in colour. The jaundice went on increasing. On the 29th, my attention was attracted by the extremely apathetic condition of the patient and his refusal to take any food or medicine. He was
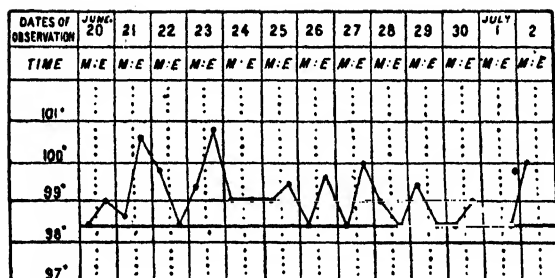
A case of acute yellow atrophy of the liver

restless and attempted to get out of his bed. In the evening he vomited several times, the vomit consisting of altered blood. He passed urine in bed clothes. The area of liver dulness was found to extend from the sixth interspace to 2 inches above the costal arch. It was evident that the patient was suffering from symptoms of malignant jaundice, and the diagnosis of acute yellow atrophy of the liver was made. On the 1st July, the patient became comatose. He was still passing urine in bed clothes, but there was no retention of urine. The dulness of the liver could with difficulty be percussed out just above the seventh rib. Examination of the urine gave the following results :—

Sp. Gravity	...	...	.. 1016
Albumen	...	. .	... Trace.
Bile	...	...	. Excess.
Tyrosin	..	...	... Crystals in large number.
Leucin	..	...	.. None
Casts	...	...	... Granular and stained with bile.
Renal epithelium	...	...	... Much.

The blood was examined for leucin and tyrosin, but the results were negative. A leucocyte count was made giving the following results :

(1) Large mononuclear leucocytes	.	11·07 per cent.
(2) Small mononuclear leucocytes	...	5·00 per cent.
(3) Polymorphonuclear leucocytes	...	83·93 per cent.
(4) Total number of leucocytes	...	7600 per. c. mm.

On the 2nd July, the liver dulness could still be made out on very careful percussion just above the seventh rib. The patient died at 11 A.M., on the same date.

Unfortunately no autopsy could be held on the case.

Remarks.—The diagnosis of the case was at first made as one of catarrhal jaundice with slight enlargement of the liver, and it was not till the patient began to show nervous symptoms that acute yellow atrophy of the liver was suspected. The nervous symptoms were associated with diminution of area of hepatic dulness.

The case is interesting as it shows that the liver may be enlarged for some time in the disease—a fact denied by many observers. Some authors, on the other hand, divide the disease into two stages :—(1) Primary stage lasting over a variable period of two days to three or four weeks or rarely six weeks (Barley) or even two months (Glynn), the liver dulness being slightly enlarged or normal. (2) Secondary stage, characterized by hæmorrhages, diminished area of hepatic dulness, the duration varying from one to seven days, or on an average from two to three days (Hunter).

Acute yellow atrophy of the liver is easily diagnosed in its second stage. Sometimes, however, the diminution in hepatic dulness comes on within a few hours before death and sometimes, again, according to some observers, this may not take place even up to the last. Trousseau goes even so far as to state that the diminution of hepatic dulness is only of minor importance in the disease. He lays greater stress on the pain in the epigastric and right hypochondrial regions. The presence of leucin and tyrosin is a very important symptom in the disease. Most observers agree with Murchison in saying that their presence " may be said to clench the diagnosis " of the disease. Osler, however, states that they may be present in afebrile jaundice with slight enlargement of the liver. They have also been noticed in abscess of the liver and also in cirrhosis of the liver and rarely in some cases of toxæmic jaundice such as phosphorus poisoning (Hunter). On the other hand, there have been cases of acute yellow atrophy of the liver in which they were absent. Out of 34 cases collected by Theirfilder, in 7 the result was negative ; in 17 both were found ; in 9 tyrosin only; in 1 leucin only. Such anomalous cases of acute yellow atrophy of the liver, in which these bodies are absent in the urine and in which the liver dulness is very little affected, may be extremely difficult to diagnose from toxæmic jaundice and also from ordinary jaundice associated with

hæmorrhages and nervous symptoms. The similarity between toxæmic jaundice and acute yellow atrophy of the liver becomes very great in those cases of the former in which the liver dulness is actually diminished, and leucin and tyrosin are found in the urine. Generally, however, the liver is enlarged in toxæmic jaundice.

Sometimes the resemblance between this disease and yellow fever is so great that some authors have gone so far as to regard the two diseases as identical (Liebermeister). Trousseaus's protest against this view is somewhat difficult to uphold. In yellow fever, however, there is generally no shrinking of the liver.

Typhoid fever, complicated with jaundice—a rare complication—may be mistaken for the disease during the first days of the attack.

The disease may sometimes have to be diagnosed when it is superadded to some other liver diseases, such as cirrhosis or chronic obstruction of the bile ducts by gallstones, and in such cases especially the liver may remain normal or be even enlarged.

The disease may occur during an occurrence of epidemic jaundice or may itself occur epidemically. The symptoms of the two are almost exactly the same, and it is very probable that in most cases they are one and the same disease.

Rarely some of the forms of æstivo-autumnal fever may be mistaken for acute yellow atrophy of the liver, but the diagnosis is easily made.

Lastly, one may mention that ulcerative endocarditis may sometimes be mistaken for the disease—a mistake which once occurred to me when I was a house physician in the Medical College Hospital, Calcutta, and Osler states that cases are on record in which one disease was mistaken for the other.

Acute yellow atrophy of the liver is one of the rarest of diseases. The rarity of the disease is attested, as Murchison says, by the fact that although a brown tongue and delirium

formerly constituted a certain passport for the transmission of all diseases to the London Fever Hospital, yet there was only one case out of 25,700 patients admitted during nine years. Dr. Stephen Smith Burt states that there is no memorandum of a single case in the Post-Graduate Hospital, New York, previous to the one described by him. In the *Transactions of the New York Pathological Society* from 1876 to 1898 there are five cases recorded. In the *Boston Medical and Surgical Journal* from 1886 to 1899 there are three cases published. Among the *Proceedings of the Pathological Society of Philadelphia* from 1857 to 1899 there are but two cases narrated. The total number of recorded cases as estimated by Hunter does not exceed 256. No case came under the observation of Osler during his varied *post-mortem* and clinical experience. "On the other hand," as he states "a physician may see several cases within a few years or even months, as happened to Reiss." I observed another case in the Medical College Hospital, Calcutta, some time ago in the wards of Lieutenant-Colonel Bomford. The case came with violent delirium and marked diminution of the hepatic dulness. The diagnosis was confirmed *post-mortem* and by microscopic examination of the liver.

A few remarks may be made about the character of the stools in this disease. Murchison states that bile is found throughout in the stools, though sometimes they are absent. Osler and Taylor state that they are generally clay-coloured containing no bile. Hunter states that they sometimes contain altered blood. In our present case the stools were pale, and in the one that occurred in the Medical College Hospital, Calcutta, the intestines contained fæces having no bile in them.

References

Allbutt's System of Medicine.
Trousseau's Clinical Lectures.
Murchison's Diseases of the Liver.
Osler's Medicine.
Lancet, December 15th, 1900.

A case of angio-neurotic œdema

A CASE OF ANGIO-NEUROTIC ŒDEMA

Definition.—"It is a disease characterized by the appearance of circumscribed swellings on different parts of the body, by preference, the face, the throat and the extremities, without apparent cause or premonition and non-inflammatory in nature."

The following case came under my observation while I was at Dacca :

Patient, æt 20, a Bengalee, came to me one morning with history of swelling of the right eyelids and the right side of his face which came on suddenly the previous night. He gave history of such attacks on a few former occasions. No history of any such disease in the family, and no history of colic or gastro-intestinal trouble associated with the attack.

On examination I found that there was a non-inflammatory œdema of the lids of the right eye and the right side of his face. There was a slight tightness complained of about the part, but there was no sign of inflammation in the œdematous part. The swelling did not pit on pressure. There were no hæmoglobinuria, albuminuria, and fever. Nothing could be traced as to the immediate cause of the condition.

The subsequent history of the case was that the œdema disappeared in about three days. He had very little complaint during this period except the presence of the swelling.

The figure is a photograph of the case taken on the day he came to me for treatment.

Diagnosis.—The diagnosis of a case of angioneurotic œdema is simple. It may have to be diagnosed from the blue œdema of Sydenham or the white œdema of Charcot as sometimes noticed in hysteria. In hysteria we are helped in our diagnosis by the presence of hysterical stigmata, hysterical paralysis, anæsthesia or contraction of the field of vision. It is barely possible to confound it with erythema nodosum, but the absence of tenderness and the pale colour will settle the diagnosis. Giant urticaria is probably a disease closely allied to angio-neurotic œdema. It is stated by Taylor that the disease is accompanied by burning, pricking and itching, but in our case they were absent and probably they are often absent. In fact Hare goes so far as to say that the absence of itching is an important difference from true urticaria. It is probable, however, that urticaria and angio-neurotic œdema are allied to each other.

Pathology.—The pathology of the disease is uncertain. It is regarded by many as a sort of vasomotor neurosis. It is thus allied to neurotic ischæmia and hyperæmia. " The œdema " Dana says " is probably similar to that which is associated with attacks of *tic douloureux* and migraine." Osler includes the various conditions, *e.g.*, simple erythema, erythema exudativum, herpes iris, erythema nodosum, certain purpuras, urticaria and angio-neurotic œdema under the category of ' erythemas.' "The essential process is a vascular change with *exudate of blood or serum*, alone or combined. While five or six of the affections just named are described usually as separate diseases, they belong to one family and are characterized by the similarity of the conditions under which they occur, the frequency with which the lesions are substituted, the one for the other, in the same patient at different times, the tendency to recurrence often through a long period of years, and lastly the identity of the visceral manifestations."

Prognosis.—The prognosis of a case of angio-neurotic œdema is unfavourable so far as complete cure is concerned.

But generally it does not much affect the general health. If it attacks the larynx it may prove fatal. In some cases nephritis may supervene and death may take place from uræmia. In others death may take place from other complications. In others again some of the graver types of diseases included by Osler under the 'erythemas' may supervene and death may take place as in the cases recorded by him.

References

Dercum's *Nervous System.*
Taylor's *Medicine.*
Hare's *Practical Diagnosis.*
Dana's *Nervous System.*
American Journal of Medical Science, January, 1901.
American Journal of Medical Science, January, 1904.

A CASE OF HÆMOPHILIA*

The patient, a young Hindu Bengalee, æt. 19, was brought to me while I was at Dacca for the treatment of recurrent attacks of swelling of the large joints, especially the elbow, from which he had been suffering for some years. The onset of these attacks was generally sudden. They were associated with fever and extreme pain over the affected joints. Besides this, he also gave history of swellings under the skin, in the various parts of the body coming on spontaneously or after slight injury. These used to disappear after some days, leaving the skin over them somewhat discoloured for a few days. On enquiry, it was found that there was a very distinct history of hæmorrhagic diathesis in his family, which could be traced through three generations (see *diagram*).

On examination of the patient, I found both the elbow joints were stiff and there was effusion into the left elbow joint which was hot and tender. The family history of the case was so very significant that I diagnosed it as one of hæmophilic arthritis. The effusion disappeared after some days. After about a month the patient had an abscess in the left gluteal region which burst of itself and it was followed by an almost uncontrollable hæmorrhage which was finally stopped by being packed with adrenalin solution and application of ice. In the course of my observation of the case which extended for several months, the patient had

* Read at the Calcutta Medical Club, 29th November, 1906.
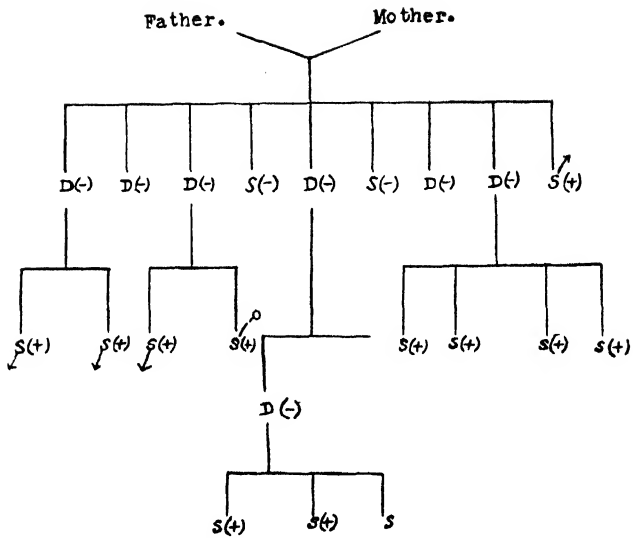
Genealogical tree of a case of hæmophilia

D = Daughter
S = Son
+ = A bleeder
− = A non-bleeder
→ − = Death from hæmorrhage

once an attack of profuse epistaxis which lasted for 2 days. He had also an attack of hæmorrhagic effusion into right ankle-joint.

Remarks —The above case is of interest, as it shows that repeated hæmorrhages into any particular joint in hæmophilia may lead to ankylosis. I do not agree that permanent deformity of a joint never takes place in hæmophilia. As regards blood examination, " A. E. Wright finds that the blood of bleeders shows great diminution in the coagulability, in several cases requiring more than an hour to clot, instead of the usual three to six minutes. A considerable diminution in the number of leucocytes and also of the blood plates, is frequently observed, facts of considerable interest, in view of the hypothesis that the formation of fibrin ferment is attributed in some measure to both of these structures. The blood-count shows a diminution in the number of red cells and hæmoglobin, roughly proportionate to the degree of the anæmia." (Bain's Text-book of Medical Practice.)

One word I should say about the treatment of hæmophilia by calcium chloride. This drug should not be given continuously to a patient suffering from hæmophilia, as, instead of increasing the coagulability of the blood, it diminishes it if continued for long periods without intermission.

ALBUMOSURIA AND THE PERIOD OF ALBUMINURIA IN CHOLERA*

The constant presence of albumose in the urine of patients suffering from cholera, after suppression has ceased, is, as far as I am aware, a new observation. Its presence was first observed by me while I was House Physician to Surgeon-General Bomford, in the Medical College Hospital, Calcutta. There is no mention of albumosuria in cholera, in all the literature on the subject, to which I had an access. There is no mention of the condition in the works of A. Lesage (Le Cholera), Liebermeister (Cholera), Rosenstein (Nierenkrankheiten), Roux (Maladies des Pays Chauds), Rho (Maliettie nei paesi caldi) and Nothnagel (System of Medicine). Among English authors, there is no mention of it in Allbutt's System of Medicine, in Manson's book on Tropical Diseases, and in other recent works on Medicine. In all the cases that I examined, albumosuria was as constant as albuminuria. In most cases the two conditions were found together but in some the albumose seemed to disappear before the albumen and in some very rare cases, the albumen disappeared before the albumose. I append here a table containing the results of examination of the urine of the cases in which this observation was made. The degrees of albumosuria and albuminuria were not proportional to each other. In a few cases there was a trace of albumose when the quantity of albumen was large.

* Read at the Calcutta Medical Club, January 17, 1907.

What is called here albumose was characterized by the following tests :—

(1) Not precipitated by heat.

(2) Precipitated by HNO_3. The precipitate was soluble on heating and re-appeared on cooling.

(3) The precipitate looked granular under the microscope and was never crystalline.

(4) The precipitate did not give the murexide test for uric acid or urates.

In a few cases the urine was treated with trichloracetic acid and picric acid separately and boiled—the filtrate in each case gave a precipitate on cooling, soluble on boiling. In a few other cases the urine was treated with equal parts of a saturated solution of NaCl and a few drops of acetic acid and boiled and filtered. The filtrate gave a precipitate on cooling which was soluble on boiling. In the case of trichloracetic acid the precipitate took 24 hours to appear.

According to Hogonneuq, there are two distinct classes of albumose : (1) Bence-Jones; this is precipitated at a temperature of $50^{\circ}C$ to $65^{\circ}C$ and then dissolves on boiling wholly or in part, and (2) the more common form of albumose which is not precipitated by heat (Epitome of Current Medical Literature, B.M.J., February 16, 1901 . The albumose that was present in cholera urine belonged to the latter variety.

Rosenstein's description of cholera urine, as given in Nierenkrankheiten, is a very complete one, which I take the liberty of quoting here (in English).

" First passed urine—small quantity (100 c c.) ; cloudy, flocculent, sometimes dark-yellow or yellowish-brown. HNO_3 gives a play of colours, resembling the Gmelin's colour reaction, only that the characteristic green colour does not generally appear. Wyss says that it is due to the presence of *indican*. In severe cases the urine is albuminous,

Sometimes, on the other hand, even after 24 hours of anuria the urine may be free from albumen. The sediment in the urine contains red and white blood corpuscles. Casts of various forms are found, hyaline and fatty and they are frequently very long; also epithelium from the kidneys. 'Mucous corpuscles and bladder epithelium are also found. Even in non-albuminous urine, casts may be found.

Re-action of the urine—faintly acid. Sp. gravity is from 1012 to 1016. The quantity of solids such as urea and chlorides are diminished; sometimes the urea is half the quantity or less. After repeated emptyings of the bladder, the quantity of urine is increased and sometimes there may be polyuria, in which urea and NaCl increase in amount. The absolute quantity of urea may now be increased though NaCl may still be below the normal. Gradually the albumen disappears and along with it the casts too. Sometimes it persists even after the albumen has disappeared."

I generally found short, fatty and granular casts in the first passed urine, while in the last stages of the albuminuria, very long hyaline casts, with granules of fat scattered through them, were found. The number of red corpuscles, found in the urine at the stage of albuminuria, was generally very small and sometimes none were found.

The duration of albuminuria in cholera, ranged from 24 to 300 hours, with an average of 12 hours, which is much greater than what has been mentioned by any previous observer. I cannot agree with Rosenstein that the urine is albuminous, only in severe cases. On the other hand I think that it is a very constant condition in cases of cholera.

No.	Albumosuria	Duration of Albumosuria	Duration of Albuminuria
1	present	36 hours	36 hours (death)
2	,,	60 ,,	60 ,,
3	,,	12 ,,	24 ,,
4	,,	12 ,,	60 ,,
5	,,	60 ,,	84 ,,
6	,,	36 ,,	36 ,, (death)
7	,,	132 ,,	132 ,,
8	,,	132 ,,	172 ,,
9	,,	300 ,,	300 ,,
10	,,	196 ,,	196 ,,
11	,,	132 ,,	132 ,,
12	,,	64 ,,	64 ,,
13	,,	120 ,,	120 ,,
14	,,	144 ,,	144 ,,
15	,,	60 ,,	60 ,,
16	,,	132 ,,	132 ,,
17	,,	..	132 ,
18	,,	......	72 , (death)
19	,,	......	60 ,
20	,,	......	36 ,,
21	,,	......	208 ,,
22	,,	......	54 ,,
23	,,	.. ...	54 ,.
24	,,	......	114 ,,
25	,,	204 hours	204 ,,
26	,,	240 ,,	240 ,,
27	,,	80 ,,	80 ,,
28	,,	64 ,,	64 ,,
29	,,	200 ,,	200 ,,
30	,,	120 ,,	96 ,,
31	,,	72 ,,	96 ,,
32	,,	66 ,,	66 ,,
33	,,	300 ,,	300 ,,
34	,,	......	.. ...
35	,,	......	......
36	,,	.. ...	......
37	,,	. ...	.. ...
38	,,	... ..	......
39	,,	......	......
40	,,	......	......

AGURIN IN THE TREATMENT OF ANURIA IN CHOLERA

Anuria is, perhaps, the most serious symptom that has to be combated in the case of cholera. In the stage of reaction, when the rice-water stools have disappeared and when the condition of the pulse has improved, the prognosis of the case still remains very grave, as long as the suppression of urine continues. We, oftentimes, find that our resources fail in the treatment of this condition and the patient dies of uræmic symptoms. I have tried *agurin* in 8 consecutive cases of cholera with very hopeful results and though my observation has still been very limited, my only justification, in publishing my results is that the drug may be given a trial by others. As far as I am aware, there are no records of cases of cholera treated with agurin for the combating of suppression of urine. I append here a table showing that agurin is a very powerful diuretic and that in every case in which I used it, the suppression of urine was relieved. In two of my cases, the treatment was begun when the patient was hopelessly ill and the symptoms of uræmia were very marked. Even, in these two cases, so far as the secretion of urine was concerned it was effected by the administration of agurin, though it was begun, perhaps, too late to lead to a cure of the uræmia.

Table showing the effect of agurin in Cholera

	Duration of anuria	Condition of patient when agurin was first administered	Dose	Time when secretion of urine began	Result
1	48 hours, before agurin was given	Stage of collapse	5 grains every 3 hours (4 doses a day)	24 hours after administration of agurin	Cure
2	48 hours, before agurin was given	Do.	Do	12 hours after administration of agurin	Do.
3	36 hours, before agurin was given	Do.	Do.	12 hours after administration of agurin	Do.
4	36 hours, before agurin was given	Do.	Do.	14 hours after administration of agurin	Do.
5	1st attack 48 hours. 2nd attack began 12 hours after secretion of urine, 1st started and continued for 24 hours when agurin was given	Patient in the state of "cholera-typhoid" with marked uræmic symptoms	Do.	12 hours after administration of agurin	Death
6	1st attack 96 hours. 2nd attack began 24 hours after secretion of urine 1st started and continued for 72 hours when agurin was given	Patient in the state of "cholera-typhoid" with marked uræmic symptoms ...	Do.	24 hours after administration of agurin	Cure
7	72 hours before agurin was given	State of collapse	Do.	8 hours after administration of agurin	Do.
8	48 hours before agurin was given	Do.	Do.	8 hours after administration of agurin	Do.

A FIVE-DAY FEVER OF CALCUTTA

In the year 1900, while working as House Physician to Surgeon-General Bomford in the Medical College, Calcutta, I made observations on a number of cases of short remittent fever, which used almost invariably to terminate on the 5th day. They were designated as cases of "Five-day Fever." They constituted a clinical entity distinct from malaria, as we never found the malarial parasites in their blood; the spleen was never enlarged and the fever used to terminate without administration of quinine, which in those cases in which it was administered, seemed to have no influence upon the course of the illness, Some of the cases came from the same house, others occurred in places where there was no other case and one occurred in the wards of the Medical College Hospital itself. Most of the cases came into the Hospital between the months of June and July, during which we observed many such cases. I append here the notes of six of these with temperature charts of three.

1. Miss B., æt. 10, admitted into Hospital on 25th June, 1900; fever without any ague fits; no cough; vomiting present; slight constipation; spleen and liver not enlarged; tongue coated; headache intense; patient's sister also suffering from the same type of fever (Chart No. I).

2. Charles, T., æt. 26, admitted into Hospital on 7th May, 1900, contracted the fever while staying in the Calcutta Medical College Hospital, where he was being treated for dysentery for more than a month. Fever without any shivering fits; spleen and liver not enlarged; lungs,

occasional rhonchi audible at the bases; tongue coated at the centre; diarrhœa present; pulse, 104 during the height of the fever.

3.   Norman, P., æt. 17, a military student of the Medical College living in the Military Students' barracks; admitted into Hospital on 12th August, 1900.   Spleen, not enlarged; extreme headache; tongue, slightly coated; no diarrhœa; number of red corpuscles—5,175,000 per c. mm.; leuco-cytes—9,231; hæmoglobin—95 per cent (Chart No. III)

4.   Mrs. R., æt. 35, fever, coming on with a feeling of cold; no vomiting or diarrhœa; no cough; spleen not enlarged; tongue coated in the centre but no prominent papillæ; headache intense; slight occasional sickness; pulse, 120 during the height of the fever; duration of fever, 120 hours nearly.

5.   Miss A., æt. 10, admitted into Hospital on 5th July, 1900; fever without any shivering; no vomiting; no cough; spleen and liver not enlarged; tongue coated in the centre with prominent papillæ and red and raw at the edges; intense headache; pulse 128 per minute during the height of the fever.   Patient's brother suffered from the same type of fever.   Fever terminated after 120 hours.

6.   McK., æt. 18, admitted into Hospital on 8th May, 1900; fever with shivering fits; extreme prostration on admission; slight vomiting and diarrhœa; spleen and liver not enlarged; lungs, nothing abnormal; tongue slightly dry and coated in the middle and red at the edges; intense headache; pulse, 64 in the convalescent state.   Duration of fever—5 days and 6 hours.

It is possible that this fever is similar to Major Rogers' 'Seven-day fever;' the slow pulse described by him in his cases was not, however, noticed in ours and the fever used to terminate on the 5th, instead of on the 7th day of illness.

I append here, however, the notes with temperature charts of 2 cases admitted in the same year into Surgeon-

General Bomford's ward which bear a close resemblance to Major Rogers' cases of "Seven-day fever."

I.   Patient, H. N., admitted on 31st August, 1900; spleen, slightly enlarged. No malarial parasites in the blood (Chart IV).

II.   Patient J K., admitted on 9th May, 1900; no rigor; intense headache; tongue thickly coated in the middle; spleen and liver not enlarged; lungs, nothing abnormal. Pulse, 56 per minute in the convalescent state. No malarial parasites in the blood. Temperature chart resembles Major Rogers' terminal cases.

Before concluding, I would point out that "Five-day" is not limited to the European and Eurasian population of Calcutta. A Bengali gentleman, was recently treated by me, in whom the fever terminated exactly on the 5th day. During his illness he was extremely prostrate; pulse was very feeble; tongue was coated in the middle with prominent papillæ; the condition resembled that of enteric for the first two or three days of the illness.

Reference

Indian Medical Gazette, Nov. 1905; *Ibid.*, March, 1906.
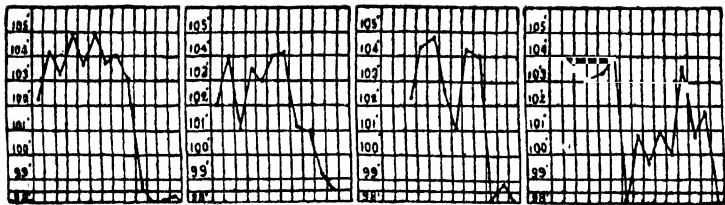
Four temperature charts of five-day fever of Calcutta

COMPENSATORY COLLATERAL CIRCU-LATION WITH "*CAPUT MEDUSÆ*" IN CIRRHOSIS OF THE LIVER WITHOUT ASCITES

Cases of cirrhosis of the liver without ascites have been occasionally met with. Such cases are of special interest, as their occurrence gave rise to the idea of the Drummond-Talma-Morison operation. Generally speaking, the collateral circulation in cirrhosis of the liver is not capable of carrying off all the surplus of blood and of re-establishing a normal vascularity and in this way removing the ascites. The latter, according to some, occurs in at least 80 per cent. of the cases. The number must be greater, when we consider those cases in which there is complete absence of hæmatemesis and melæna which frequently vary inversely with the amount of ascites present. The following case is of considerable interest showing a perfect collateral circulation without the least amount of ascites and without any hæmatemesis and melæna.

The patient, a Bengalee, aged 35 years, was first examined by me about four years ago for treatment of enlarged spleen. He gave a history of attacks of fever resembling malaria. There was no history of hæmatemesis, melæna, or jaundice. On examination the superficial veins of the abdomen were found enormously enlarged, especially the right epigastrics, which were tortuous and very prominent (Fig. 1). The veins round the umbilicus were somewhat dilated. There was a mark of a scar in the right hypochondral

region—the result of an operation in the Medical College Hospital, Calcutta, for an inflamed thrombus in the dilated veins situated here. The spleen was found enlarged, extending about two inches below the costal arch. The liver dulness extended from the seventh intercostal space to one inch below the costal' arch. The left lobe of the liver felt hard and rough. There was 'no ascites. A loud systolic bruit was audible over the right superficial and superior epigastric veins. A systolic bruit was also audible at the pulmonary area of the heart. The patient was somewhat anæmic and had some evening pyrexia. Digestion was good. The urine was scanty and high-coloured. The patient was under my observation for some months. The fever gradually disappeared, the appetite increased, and the only symptom which afterwards he complained of was the presence of the enormously dilated veins. Since then I have seen the patient from time to time improved in general health and without any ascites.

The patient came to me the last time in March, 1907, complaining of the enlargement of the veins in the abdominal wall, which were a source of inconvenience to him and which were getting so progressively enlarged that he feared they might rupture. He asked for their complete removal by surgical interference. He looked better in health than when I saw him, about four years previously. He was only slightly anæmic, the systolic bruit at the pulmonary region of the heart having completely disappeared. The spleen extended two inches below the costal arch. The liver dulness was smaller than before and the edge of the liver was not felt below the costal arch. The dulness could just be made out in the seventh intercostal space in the right mammary line. There was no ascites. The patient had no fever and his appetite was good. The following was the condition of the venous anastomosis in the abdominal wall (Fig. 2). 1. The right superficial epigastric was enormously
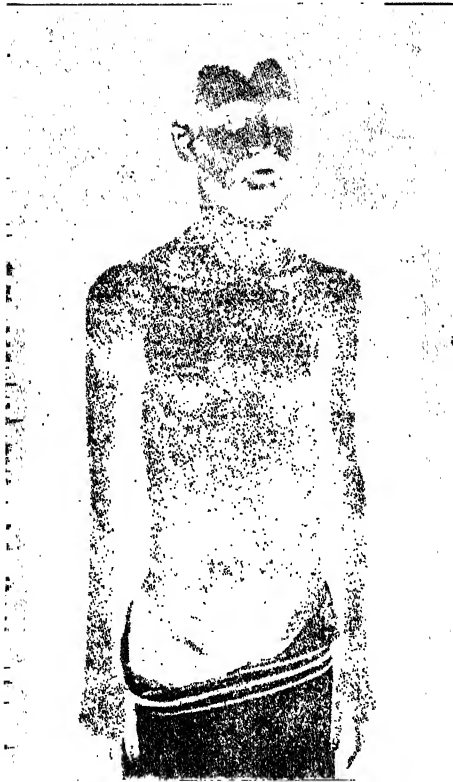
Cirrhosis of the liver without ascites

dilated, looking almost like coils of intestines and anasto-mosing with the superficial branches of the superior epigas-tric which were also very much enlarged. 2. The right superficial epigastric anastomosed directly with the branches of the long thoracic which was also very prominent and enlarged. 3. The left superficial epigastric was more di-lated and the anastomosis between it and the left superior epigastric more prominent than when observed about four years previously. 4. The presence of dilated veins round the umbilicus gave rise to a well-marked "caput medusæ."

One point that struck me was the fairly good health which the patient was enjoying during all these years. Could the complete absence of ascites account for this fact? Ascites may do harm in more ways than one:—1. By pressing upon the vena cava it may interfere with the venous circula-tion of the lower extremities, giving rise to œdema in these parts. 2. By pressing upon the renal vessels and kidneys. 3. By setting up a vicious circle by pressing upon the branches of the portal vein and thus interfering with the functions of the stomach and the intestines and also offering more obstruction to the portal system. 4. By pressing upon the diaphragm and interfering with respiration and cardiac action. 5. By abstracting from the blood a large quantity of albuminous fluid.

It remains for me to give a brief account of the collateral circulation which may be developed in cirrhosis so as to enable the blood of the portal system to reach the systemic veins. 1. Hæmorrhoidal branches of the inferior mesen-teric anastomosing with those of the internal iliac. 2. Mesen-teric veins anastomosing with those of the abdominal wall. 3. Coronary veins anastomosing with the œsophageal veins and thus communicating with the azygos veins. 4. Coronary veins and veins of Glisson's capsule on the one hand and the phrenic veins on the other. 5. One or more veins in the round ligament (the accessory portal vein or parumbilical vein

or rudimentary umbilical vein of different authors) connecting the portal with the epigastric and other veins of the abdominal wall. 6. Veins of the pancreas, the duodenum, the colon, and the rectum communicating with the retroperitoneal branches. 7. Veins lying in the subperitoneal tissue, between the folds of the hepatic ligaments and the peritoneal folds round the liver communicating with the portal system on the one hand and the phrenic veins and azygos major on the other (the minor accessory portal veins of Sappey). Many of these penetrate the capsule of the liver. 8. Left renal veins and the veins of the intestines, especially those of the colon and the duodenum. 9. Superficial branches of the portal veins of the liver and the phrenic veins. 10. Very rarely communication between the parumbilical vein through an abdominal vein with the right illiac vein. Besides the foregoing there may be innumerable new vessels that may develop from adhesions.

I shall 'mention here another cause besides the collateral circulation mentioned above which may prevent the occurrence of ascites, namely the increased exudation of fluid into the peritoneal cavity may be reabsorbed by the lymphatics of the diaphragm and the parietal peritoneum, which are independent of the portal system.

References

Crit. Med. Jour., September, 1896.
Nothnagel's Encyclopædia of Practical Medicine (Diseases of the Liver, etc)
Allbutt's System of Medicine, Vol. IV.
Sappey : Centralblatt fur Chirurgie, 1901.
Drummond: Brit. Med. Jour., February, 1889.
Quincke : Ascites, Deutsches Archive fur Klinische Medicin, 1882.

THE BACTERIOLOGY OF THE BLOOD AND THE TREATMENT OF INFLU-ENZA OCCURRING EPIDEMI-CALLY IN CALCUTTA

Cases of influenza admitted in the wards of one of us (U. N. B.) in the Campbell Hospital from October to the middle of December, 1918, numbered 374. The blood of the patients for making cultures was taken from the veins at the bend of the elbows, and two to three c.c. were immediately put into broth and agar tubes for culture. In ten cases the blood was taken from the heart under perfectly aseptic conditions within half to one hour after death.

The number of cases whose blood was taken for culture amounted to 90. The cases were clinically divided under two heads : (1) Mild cases in which no pneumonic symptoms were present, and (2) cases in which pneumonic symptoms were present and were regarded as severe cases.

The results of blood culture of these two types of cases were very characteristic. Thus, out of 14 mild cases only one showed the presence of streptococci in the blood, and this case subsequently developed pneumonic symptoms and died. On the other hand, out of 76 severe cases, 36 showed the presence of streptococci or pneumococci in the blood.

The blood cultures in positive cases generally showed the presence of streptococci or pneumococci. In two, there was pure culture of staphylococcus aureus obtained. In two, Gram-negative, capsulated cocci which formed no growth on agar or subculture were obtained. In four, bacteria,

somewhat resembling typhoid bacilli, but Gram-positive, were obtained.

Summarizing the results of the blood examination, we have as follows :—

Out of 90 cases—

23 showed streptococcic infection of the blood.

14 showed pneumococcic   do.   do.

2 showed the presence of pure culture of staphylo-coccus aureus.

2 showed the presence of capsulated Gram-negative cocci.

4 showed the presence of a Gram-positive motile bacillus somewhat resembling typhoid bacillus.

It will thus be seen that the micro-organisms most commonly present in the blood were either pneumococci or streptococci.

Pneumococci   ...   ... 16 per cent nearly.

Streptococci   ...   ... 26 ,,   ,,

Leaving the mild cases out of consideration we have pneumococci present in 18 per cent and streptococci in 27 per cent of the severe cases. The mild cases showed no bacterial infection of the blood.

The streptococci found could be distinguished as follows :—

(1) Markedly hæmolytic   13

(2) Slightly hæmolytic   2

(3) Non-hæmolytic   8

In some, streptococcus brevis was found. In most of the cases streptococcus longi were found.

In 10 cases of the pneumonic type of the disease, cultures were made from the blood of the heart soon after death with the following results :—

(1) One showed pneumococci.

(2) Three showed streptococci without hæmolytic properties.

(3) One showed a hæmolytic streptococcus.

(4) One showed the presence of a Gram-positive motile bacillus otherwise resembling typhoid.

SEROLOGICAL TESTS

We have done a limited number of agglutination tests with the strains of streptococci obtained, and also with the anomalous type of bacilli above described, and the following results were obtained : —

Case No.	Result of blood culture.		Agglutination test.		Nature of case clinically
65	No growth	..	No clumping with any strain of streptococci obtained.		Mild case.
80	Do.	...	Do.	...	Do.
82	Do.	...	Do.	...	Severe case.
84	Do.	...	Do.	...	Mild case.
60	The motile Gram-positive bacilli.		One strain of streptococci showed clumping (1 in 20), partial (1 in 40). The bacilli showed clumping (1 in 40).		Severe case.
71	Streptococci		The strain of streptococci obtained showed clumping (1 in 40).		Do.
75	Do.	...	Do.	...	Do.
76	Do.	...	Clumping (1 in 20)	...	Do.
81	Pneumococci	...	No clumping with any streptococci obtained. Clumped with the Gram-positive bacilli (1 in 20).		Severe case.
83	Do.	...	Do.	...	Do.

INOCULATION EXPERIMENTS

A 24 hours' culture of one of the strains of streptococci with hæmolytic properties, obtained from the blood of one of the patients, was injected subcutaneously into one of two guinea-pigs kept inside the same cage. The inoculated guinea-pig died after 60 hours. Smears were made of the spleen, the heart blood, and the lungs. In every one of these the same kind of streptococci was obtained as that with

which the guinea-pig was inoculated. The accompanying diagram was obtained from a smear of the spleen of the inoculated guinea-pig. The second guinea-pig seems to have also been infected by contact (?) with the inoculated one. The second died 3 days after the death of the inoculated one, and the same strain of streptococci was obtained in the organs as in the inoculated one. Further experiments are being made to determine the infectivity of this strain of streptococci. A portion of this paper was read before the Calcutta Medical Club in December, 1918.

TREATMENT

Remarks by Dr. Brahmachari

Before discussing the treatment of influenza I adopted in my wards, it would be interesting to study the prognosis of this disease as determined from the statistics of admissions and deaths.

Taking all the admissions into account, there were 141 deaths out of 374, i.e., 37 per cent nearly. Leaving the cases that died within 24 hours, the death-rate was nearly 25 per cent. There was no death among the mild or non-pneumonic cases. The death-rate among the pneumonic cases was nearly 67 per cent. Leaving the cases that died within 24 hours after admission, and those that were treated with the special treatment to be subsequently mentioned, the percentage of death-rate among the remaining 85 cases was nearly 82 per cent—an appalling figure when compared with the death-rate of pneumonic cases admitted into my ward in former years. Thus, some years ago I collected the statistics of death-rate of cases of lobar pneumonia, admitted in my wards in the Campbell Hospital, and it was found that the death-rate was only 26 per cent. We thus conclude :—

(1) Recovery among ordinary cases of lobar pneumonia   ... 74 per cent
(2) Recovery among cases of influenza complicated with pneumonia 18 per cent

These are appalling figures and any medicaments that would reduce this terrible death-rate must be of the greatest value. Various drugs were tried by me, some of which I shall mention very briefly. These ,include mercury in the form of electro-mercurol, silver in the form of electro-argol, colloidal manganese and stannoxyl. None of 'the drugs seems to have influenced the course of the disease.

Among the treatments that seem to have influenced the course of the disease in severe cases are the following :—

(1) Vaccines.

(2) Colloidal iodine.

(3) Formaldehyde sodium-bisulphite.

1. *Vaccines* :—

Forty cases were treated with injections of combined catarrhal vaccine of P. D. & Co. Half c.c. was injected at the first dose, and another half on the 4th or 5th day. Leaving the mild cases included among these, the percentage of death-rate was nearly fifty per cent.

Cases which showed pneumococci in the blood were treated with the combined pneumococcus vaccine of P. D. & Co. A few cases were treated with anti-streptococci serum. The number of cases treated with these was not sufficient enough to justify any conclusions. A vaccine from the different strains of streptococci obtained from the heart and the veins has been prepared and used in a few cases.

2. *Treatment with intravenous injection of iodine* :—

I have used colloidal iodine in the form of collosol iodine of Crookes (1 in 500). Doses of 10 c.c. to 30 c.c. have been given intravenously. This would be equivalent to 1/3 to 1 grain of pure iodine. No untoward results followed these intravenous injections. The injections were given once a day. Altogether 5 to 6 injections were given

in each case. The results seem to be satisfactory. Thus, out of 21 severe cases of influenza with pneumonic symptoms, 8 died, giving a recovery of 62 per cent of the cases. This compares very favourably with the death-rate of 82 per cent among the untreated cases.

3. Formaldehyde-sodium-bisulphite :

Fifty to 100 c.c. of 1 in 1,000 solution, in normal saline, were given intravenously in a series of 19 cases of pneumonia with influenza. The number of injections ranged from 3 to 5, being given once a day or sometime on alternate days, six out of 19 cases died, giving a recovery of 68 per cent of the cases.

I append here the notes of one of the worst cases of influenza-pneumonia, apparently cured by intravenous injection of colloidal iodine.

Patient, aet. 65, developed broncho-pneumonia after influenza. For a few days her condition was almost desperate, the pulse being kept up by injection of strychnine and digitalin and caffeine-sodi-benzoas. There was an extensive patch at the base of the right lung. There were also patches of broncho-pneumonia in the left lung. The patient was given 6 injections of colloidal iodine and recovered.

I am indebted to Colonel Leventon, I.M.S., Superintendent, Campbell Medical School, for giving me every facility in carrying on my researches, and to Dr. Surendra Nath Ghosh, Bacteriologist, Presidency General Hospital, for the valuable help he has given me in the bacteriological portion of this investigation. In our opinion, whatever may be the part played by the influenza bacillus in the etiology of this disease, the high death-rate is due to streptococcal and perhaps pneumococcal infection.

ANTIMONY IN FILARIASIS.

To

The Editor of THE LANCET.

Sir,

I read with keen interest Sir Leonard Rogers' paper entitled " Preliminary Report on the intravenous injection of Antimony in Filariasis," published in your journal of Oct. 4, 1919.

In my observations on the use of antimony in this disease in my book " *Kala-azar, its Treatment* "—published by Butterworth and Co. (India), in February, 1917, and reviewed in *The Lancet* of Nov. 10, 1917—the following words occur on p. 115 : " It is difficult to determine the effect of any drug in filariasis, as the disease may remain latent for prolonged periods. Several cases have been treated with intravenous injection of tartar emetic and antimonyl sodium tartrate. In one case, however, there was a very marked reduction of the parasites in the peripheral blood and the fever stopped. In another case there was apparent disappearance of the embryos from the peripheral blood."

I am very glad to find that Sir Leonard Rogers' observations are corroborative of mine. My further observations will be published when more definite results are obtained.

I am,
Sir,
Yours faithfully,

Calcutta, Nov. 12, 1919.   U. N. BRAHMACHARI.

TREATMENT OF CEREBRO-SPINAL MENINGITIS BY SPINAL IRRIGA-TION WITH ELECTRARGOL

Some years ago Widal reported a desperate case of cerebro-spinal meningitis which was apparently cured by washing the spinal theca with solution of collargol. Bouche and his colleagues have recorded a case in which the spinal theca was washed with warm sterile normal saline solution through two needles, one introduced between the 2nd and 3rd lumbar vertebræ and the other between the 7th cervical and the 1st, thoracic, but no improvement followed the operation and the patient died after a month later. Halaham reported successful cases treated by washing the spinal theca with half per cent solution of carbolic acid in normal saline combined with lumbar puncture and injection of meningo-coccus serum.

The success in Widal's desperate case led me to try spinal irrigation with electrargol in some cases of the disease. Nine cases were treated in the above way, out of which four were very severe ones.

The method adopted for spinal irrigation was as follows :—

Lumbar puncture was generally made between the 2nd and 3rd lumbar vertebræ and 1 to 2 oz. of cerebro-spinal fluid taken out in the usual way. A dilute solution of electrargol (1 in 10) in normal saline was gently introduced into the spinal theca by means of a 5 c.c. Record syringe. After this

the foot of the bed was kept raised for two to three minutes and then lowered and the fluid allowed to come out through the puncturing needle. Each time about 15 c.c. of the dilute electrargol solution were introduced and after allowing the same amount of fluid to come out through the needle, another 15 c.c. were introduced. In this way, the spinal theca was washed with nearly 100 c c. of the dilute electrargol solution. A small amount of electrargol solution was allowed to remain inside.

The irrigation was generally performed every 3 or 4 days, depending upon the effect of the previous operation upon the symptoms present. No untoward results followed, except in two of the cases, in which the operation was followed by a sharp rigor each time the washing was performed.

All the cases showed the presence of meningococcus in the cerebro-spinal fluid.

The effect of the treatment in the cases that recovered consisted of the amelioration of the symptoms, as shown by fall in the temperature, diminution in the rigidity of the muscles and in the delirium or the coma that was present in the cases. In one case, the fluid, which was found turbid after the first puncture, became almost clear after the second irrigation.

The cases treated in the above way compare very favourably with those treated with intra-thecal injection of meningococcus serum or frequent removal of the cerebro-spinal fluid by repeated lumbar puncture. From the statistics appended here, it will appear that very satisfactory results were obtained in the cases that were treated with spinal irrigation.

In my opinion the combination of spinal irrigation with non-irritating antiseptics of the nature of collargol or electrargol with intrathecal injection of meningococcus serum after lumbar puncture should prove advantageous in the treatment of cerebro-spinal meningitis.

It may be mentioned here that in one case the spinal theca was at first washed with a solution of formaldehyde-sodium bisulphite (1 in 500) in distilled water without any untoward results.

Statistics of cases of cerebro-spinal meningitis treated in different ways :—

Total number = 31 ; death = 16 ; death rate = 51·5 per cent.

(1) Cases treated with or without intrathecal injection of meningococcus serum after lumbar puncture = 7 ; 4 died: death rate = 57·1 per cent.

(2) Cases treated with liq. hydrag. perchlr. and potass. iodide rwithout lumbar puncture = 15 ; 10 died ; death rate = 66·6 per cent

(4) Cases treated with spinal irrigation with electrargol after lumbar puncture = 9 ; 2 died ; death rate = 22·2 per cent.

I append here in brief the notes of four of my cases treated by spinal irrigation :

(1) Patient, æt. about 16, was admitted in my ward on 26. 6. 1919, in an unconscious state. Temperature 103°F. Marked stiffness of the neck and Kernig's sign present; pulse—146 on admission ; lumbar puncture was performed four times and each time about 1 oz. of thick turbid fluid containing meningococcus was drawn out. There was, however, no amelioration of the symptoms. After the fourth puncture, the theca was irrigated with electrargol which was repeated three times. Patient recovered and left hospital on 11. 8. 19. (*See* Chart No 1).

(2) Paient, S., æt. 16, Hindu male, was admitted in my ward on 21. 6. 1918 in an extremely low condition ; pulse—146, of low tension ; eyes—congested ; neck—stiff. Marked restlessness and profuse perspiration present. The patient was semi-conscious and screamed out from time to time due to intense headache which became worse towards the
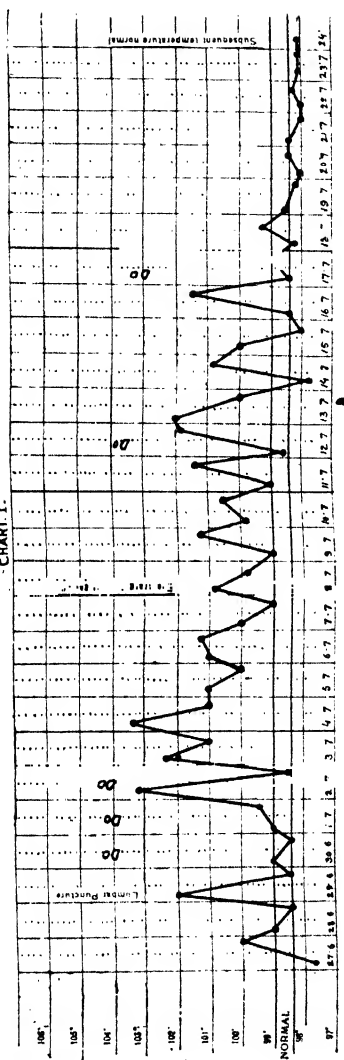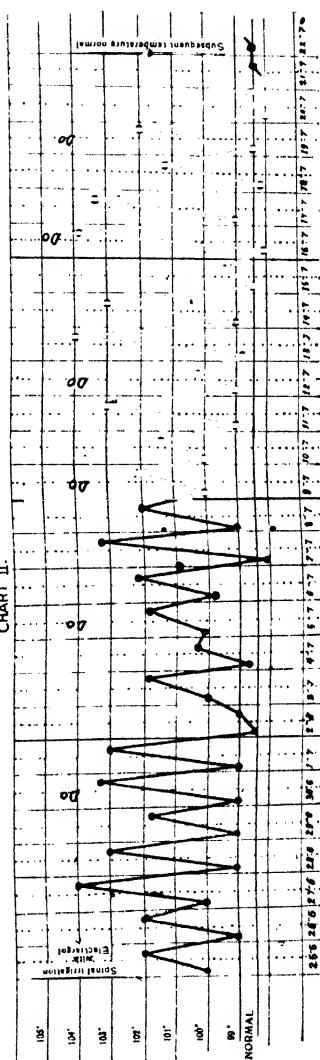
Temperature charts of cases of cerebro-spinal meningitis treated with spinal irrigation of electrargol

CHART. III
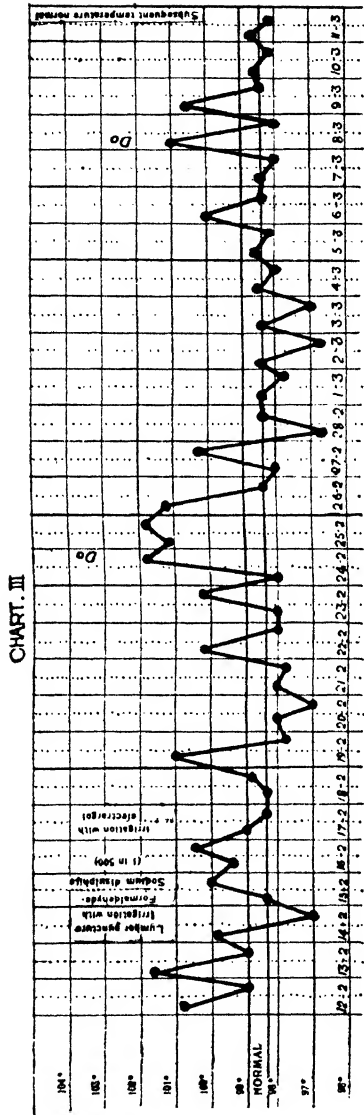
Temperature chart of a case of cerebro-spinal meningitis treated by spinal irrigation with electrargol

evening. After admission into hospital his condition became worse, Kernig's sign became very marked and retraction of the neck increased. Irrigation with electrargol was performed for the first time on 29. 6. 18. The patient recovered and was discharged from hospital on 2. 8. 18. (Chart II.)

(3) Patient, M., æt. 30, Mahomedan male, was admitted on 11. 2. 1919, in a semi-conscious state with marked restlessness and stiffness of the neck; pulse—140 and respiration—40 per minute. Bladder found distended at the time of admission. Kernig's sign and retraction of the neck—marked.

The spinal theca was at first washed with solution of formaldehyde-sodium bisulphite in normal saline (1 in 500) after removal of 2 oz. of cerebro-spinal fluid by lumbar puncture. This was not followed by any amelioration of the symptoms and subsequently spinal irrigation with electrargol was performed. Patient recovered and was discharged from hospital on 15. 3. 19. (Chart III.)

(4) Patient, S., æt. 30, Hindu male, was admitted on 11. 1. 1918. in a comatose condition. Neck—stiff, eyes—congested, Kernig's sign—marked. Lumbar puncture was performed on two successive days followed by spinal irrigation with electrargol. Each time about 1 oz. of turbid fluid was drawn out. Patient died on the 4th day after admission and one day after spinal irrigation was performed for the second time.

A CASE OF TYPHOID FEVER WITH UNUSUAL SYMPTOMS AND WITH DETAILED POST-MORTEM AND BACTERIOLOGICAL FINDINGS

Patient, A. S , Hindu male, æt. 25, a postal peon by occupation, was admitted in my ward in an unconscious state on 7. 8. 1924. Patient's relatives stated that he had fever for seven days and became unconscious on the seventh day of his illness.

Condition on admission : Unconscious; pupils dilated; neck stiff; lock-jaw present. There was stiffness of the muscles on the back and the lower extremities. Knee-jerks were increased being more marked on the left side.

There was such a marked lock-jaw and so much stiffness of the muscles of the back and the extremities, that the condition simulated tetanus. The unconsciousness together with the above-mentioned symptoms led to the provisional diagnosis of cerebro-spinal meningitis or cerebral malaria.

Nothing abnormal was audible in the heart. There was feeble breathing at the bases of both the lungs.

Blood Examination—

W. B. C.	...	5,600
R. B. C.	...	5,200,000
Hb.	...	80%
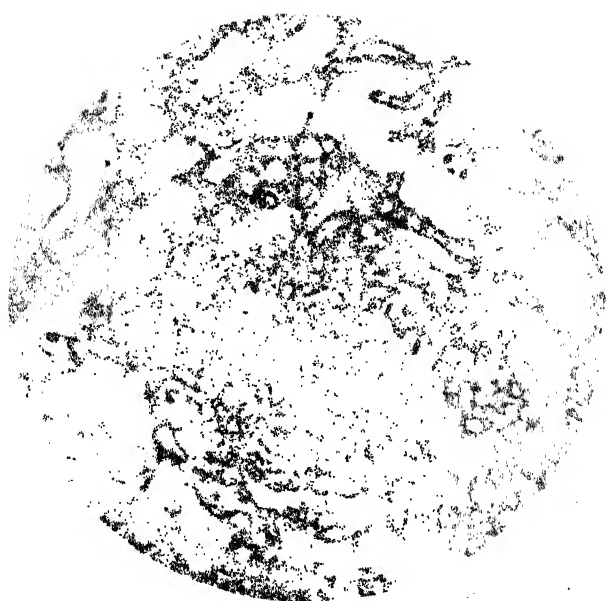
Section of liver showing focal necrosis

Lower ileum showing congestion and slight swelling of Peyer's patche

Widal reaction—positive for typhoid (1 in 25), negative with higher dilutions, negative for para-typhoid A and B in all dilutions.   No malarial parasites.

Blood culture—not made.

Lumbar puncture—not made.

Patient died about 24 hours after admission.

Post-Mortem findings

Report by Captain Shanks, I. M. S., Professor of Pathology, Medical College, Calcutta :

"Anatomical Diagnosis—

(1) Pleuritis with sero-fibrinous effusion, bilateral.

(2) Acute congestion and œdema of both lungs.

(3) Congestion and slight swelling of Peyer's patches in lower ileum (see diagram).

(4) Congestion and enlargement of spleen.

(5) Congestion and enlargement of mesenteric lymph nodes.

(6) Acute hæmorrhagic nephritis (see diagram).

(7) Hæmorrhage in left supra-renal gland.

(8) Acute congestion of meningeal blood vessels, with an increased amount of serous (non-purulent) fluid in the lateral ventricles.

Bacteriological findings

" Cultures from lungs, kidneys, spleen, Peyer's patches, gallbladder, serous fluid in lateral ventricle and mesenteric lymph nodes—all showed pure growth of typhoid bacillus.

" The case, from the findings in the brain, corresponds with those of the second group showing meningeal features as described by Osler (the so-called serous meningitis in typhoid fever).

Besides the above, there was presence of focal necrosis in the substance of the liver (see diagram).

Remarks—The case is very interesting on account of the unusual symptoms presented by it. The partial Widal reaction was no doubt due to the blood having been examined before sufficient agglutinins had been developed. Hæmorrhagic nephritis was an interesting lesion. There is nothing in the bacteriological findings to show the presence of a secondary infection.

Acknowledgment
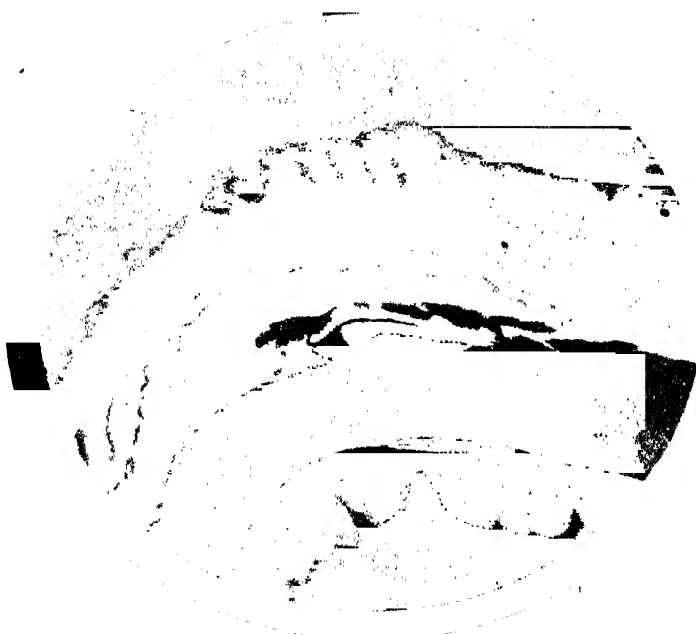
Section of small intestines showing round-celled infiltration
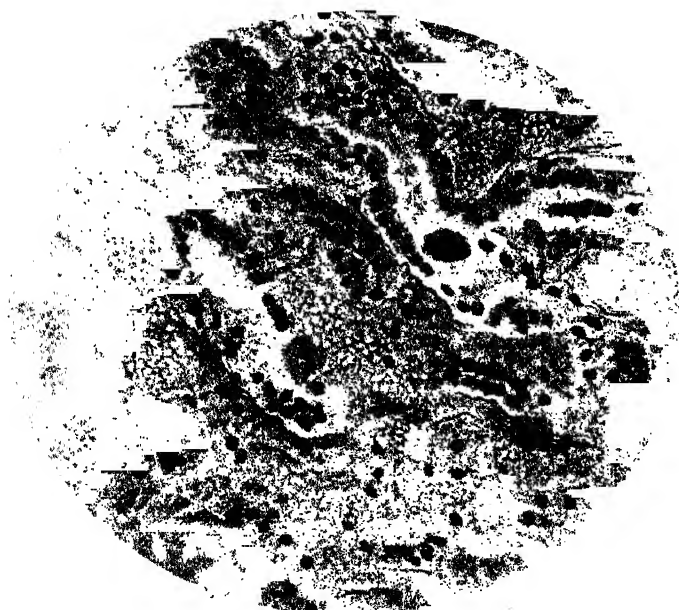
RESEARCHES ON BLOOD-SUGAR IN INDIANS *

Part I

Blood-sugar observations in young people of Bengal

Diabetes being a common disease in Bengal, observations on sugar tolerance among the young people of Bengal to determine any defect in their sugar tolerance are of paramount importance. The following observations show that this defective sugar tolerance is manifested in a definite percentage of students in Bengal.

Our observations were mostly confined to Bengali students studying in the Calcutta Medical College, living mostly on a diet which is characterized by high carbohydrate value. We also had the opportunity of examining the blood-sugar of some strict vegetarians. The age of most of the students under our observation ranged from 19 to 26 years and in a few from 26 to 30 years. No selection was made among the students, but the blood of any one who volunteered was examined under one *common* condition which consisted of starvation for 10 hours in every case.

The blood-sugar was estimated by Maclean's volumetric method. In a few cases we compared our results with those obtained by Calvert's colorimetric method of sugar estimation (*Biochemical Journal*, 1924) using the latest type of colorimeter—the Klet-Kober Colorimeter—and the difference was found to be slight, as was also observed by Calvert. Our method may be briefly described as follows :

Instead of taking the blood from the finger we invariably took the blood from a vein at the bend of the elbow, from

which 0·2 c.c. was measured by a Maclean's pipette. Immediately after the blood was drawn, the subject was given to drink 50 grammes af glucose dissolved in 150 c.c. of water or very weak tea. After this the blood was taken every half hour and examined till the sugar content dropped to the original level. In this way the blood-sugar concentration in every individual was estimated at different intervals after administration of glucose and the curves thus obtained are shown in the accompanying tables.

From a study of these curves which show our observations on 47 cases, we observed the following :--

(1) As regards the initial sugar-content of blood after 10 hours' starvation, we found that there was a wider variation among the subjects examined than is found in healthy Europeans. The figures ranged from 0·075 per cent to 0·135 per cent after 10 hours' starvation.

(2) Among 47 cases there was only one case in which the original sugar level was reached in one hour after taking 50 grammes of sugar (Case No. 32). In twenty-nine cases the original level was reached in one and half hours. In twelve cases the original level was reached in two hours. Five cases were found to be deficient in sugar tolerance, that is 10·0 per cent were found to be potentially diabetic.

(3) Kidney threshold.

In the case of Europeans the normal renal threshold for excretion of sugar is reached when blood-sugar is approximately 0·18 per cent and ' the great majority of healthy people tend to have glycosuria whenever the blood-sugar approaches the region of 0·2 per cent ' (Maclean). Our observations on ' kidney threshold ' for sugar in the case of Indians in the present investigation was limited, as we examined the urine for sugar in 15 cases only before and after sugar administration. Out of seven cases in which the blood-sugar rose higher than 0·18 per cent, no sugar was detected in the urine in one.

(4)   Among 47 cases examined, there were 8   pure vegetarians.   Of these one case showed   defective · sugar tolerance,. but in none of the remaining 7 did the sugar concentration rise as high as 0˙18 per cent.   Among the pure vegetarians whom we had the opportunity to examine, there were 5 Beharis and 3 Bengalis.

(5)   Among 39 cases who lived on a mixed diet there were 4 which showed defective sugar tolerance.   These are *potential diabetics*.   In one case there was a trace of sugar in the urine after sugar administration, though the sugar concentration curve in the blood was fairly normal.

Observations

Defective sugar tolerance is manifested among many medical students in Bengal living on a dietary containing a large amount of carbohydrates.   They are *potential diabetics*.

TABLE I.   (Mixed diet)

Percentage of sugar in the blood at different intervals after administration of 50 grammes of glucose

	½ hour	1 hour	1½ hours	2 hours
1	·075	·125	·116	·082	·076
2	·085	·123	·091	·089	
3	·093	·134	·106	·092	
4	·096	·132	·118	·094	
5	·096	·150	·124	·103	·096
6	·098	·140	·112	·096	
7	·100	·146	·122	·098	
8	·105	·170	·145	·104	
9	·106	·160	·130	·104	
10	·110	·168	·138	·106	
11	·110	·170	·163	·141	·109
12	·110	·170	·150	·115	·104

TABLE I. (Mixed diet)—*contd.*

		½ hour	1 hour	1½ hours	2 hours
13	·113	· ·158	·134	·108	
14	·113	·166	·142	·112	
15	·115	·170	·164	·114	
16	·115	·177	·156	·122	·110
17	·115	·176	·140	·115	
18	·116	·168	·138	·114	
19	·117	·180	·176	·140	·115
20	·117	·180	·156	·116	
21	·117	·173	·187	·168	·119
22	·118	·176	·154	·117	
23	·118	·180	·168	·136	·116
24	·118	·177	·158	·120	
25	·118	·178	·150	·120	
26	·119	·198	·152	·120	
27	·120	·206	·196	·187	
28	·123	·182	·150	·118	
29	·124	·182	·170	·170	·143 D
30	·125	·196	·165	·135	·122
31	·126	·190	·160	·126	
32	·126	·178	·126		
33	·126	·212	·196	·161	·132 D
34	·126	·196	·142	·126	
35	·131	·206	·168	·152	·142 D
36	·132	·193	·240	·179	·143 D
37	·132	·206	·154	·132	
38	·132	·147	·177	·137	·130
39	·135	·184	·170	·158	·132
Average of the total	·1144	·167	·1514	·119	·1151

D--Potential diabetics

TABLE II.   (Pure vegetarians)

Percentage of sugar in the blood at different intervals after administration of 50 grammes of glucose

		$\frac{1}{2}$ hour	1 hour	$1\frac{1}{2}$ hours	2 hours
1	·096	·158	·099	·096	
2	·103	·152	·135	·104	
3	·106	·166	·173	·135	·104
4	·112	·115	·124	·112	
5	·112	·158 .	·124	·112	
6	·115	·177	·150	·116	
7	·120	·149	·135	·120	
8	·126	·196	·186	·160	·138 D
Average of the total	·1112	·1576	·142	·118	·1115

D—Potential diabetics

RESEARCHES ON BLOOD-SUGAR IN INDIANS

Part II

In the *Indian Journal of Medical Research* (October, 1925), we have shown that five of forty-seven cases among the student community of Bengal were potential diabetics, as determined by the sugar tolerance tests of their blood. We have subsequently observed that out of a total number of forty-three students whose urine was examined after administration of 50 grammes of glucose, five passed sugar in the urine. The number corresponds fairly to the number of potential diabetics determined by sugar tolerance test of the blood. In the present investigation for determining the renal threshold for sugar the individuals chosen were from the same class of men, consisting of Bengalee medical students.

The plan that has been followed in our cases for determining the renal threshold is as follows :

The individual's blood and urine were examined in the morning, no food having been taken after the last dinner the previous night. About 9 a.m. he was given a drink of water containing glucose. Two series of cases were made. To one series, 50 grammes of glucose were administered. To the other, 150 grammes or more were administered. The object of giving different amounts of glucose was to determine how far our observations were in agreement with those of MacLean who found that 50 grammes, or 100 grammes, or more had the same effect on the height of the blood-sugar curve—a point on which we shall not, however, enter into any discussion in the present paper.

First Series of Cases

Amount of glucose given—50 grammes

Blood-sugar before glucose administration	After administration of glucose				Examination of urine
	$\frac{1}{2}$ hour	1 hour	$1\frac{1}{2}$ hours	2 hours	
	%	%	%	%	%	
1 ·117	·173	·187	·168	·119	*Nil* before sugar was given and present after it was given.
2 ·125	·184	·170	·158	·132	Do. do.

Amount of glucose given—150 grammes or more

	15 minutes after taking glucose		30 minutes after taking glucose		45 minutes after taking glucose		60 minutes after taking glucose		75 minutes after taking glucose		Grammes of glucose taken
	Blood	Urine	Blood	Urine	Blood	Urine	Blood	Urine	Blood	Urine	
	%		%		%		%		%		
3	·183	No sugar.	·164	Passed no urine.	·151	Very slight trace.	·158	Passed no urine.	·158	Trace	75
4	·173	Very slight trace.	·188	Trace	·177	Sugar present	·141	Trace	·125	Trace	180
5	·184	Trace	·169	Present.	·179	Present.	·157	Present.	·166	Present.	180
6	·179	No sugar.	·169	Very slight trace.	·154	Very slight trace.	·142	Trace	·146	Trace	180
7	·162	Do.	·169	No sugar.	·166	No sugar.	·152	Slight trace.	·149	Trace	180

Therefore the renal threshold was not, in these cases, above ·187, ·184, ·183 ·188, ·184, ·179 and ·169 respectively, or, on the average, ·182 per cent.

SECOND SERIES OF CASES

Amount of glucose given—50 grammes

	Blood sugar before glucose administration	After administration of glucose				Examination of urine
		½ hour	1 hour	1½ hours	2 hours	
	%	%	%	%	%	
8	·110	·170	·163	·141	·109	No sugar before or after administration of glucose
9	·106	·166	·173	·135	·104	..
10	·132	·147	·177	·137	·130	..
11	·124	·182	·170	·170	·140	...
12	·126	·178	·126	...	..	...

Amount of glucose given—180 grammes

	15 minutes after taking glucose		30 minutes after taking glucose		45 minutes after taking glucose		60 minutes after taking glucose		75 minutes after taking glucose		Grammes of glucose taken
	Blood	Urine	Blood	Urine	Blood	Urine	Blood	Urine	Blood	Urine	
	%		%		%		%		%		
13	·163	No sugar.	·138	Passed no urine.	·149	Passed no urine.	·139	Passed no urine.	·126	Sugar nil.	180
14	·128	No sugar.	·143	No sugar.	·119	No sugar.	·119	No sugar.	·119	No sugar.	180

Therefore the renal threshold was not, in these cases, below ·170, ·173, ·177, ·182 ·178. ·163 and ·143 respectively, or, on the average, ·169 per cent.

We may thus conclude that the renal threshold in the case of young healthy Bengalee students was $\dfrac{\cdot182 + \cdot169}{2}$ or

·175 per cent which fairly corresponds to the European standard.

In a few cases, the renal threshold was apparently high, and these cases we describe separately below :

Blood-sugar value before administration of glucose		After administration of 50 grammes of glucose				Examination of urine
		½ hour	1 hour	1½ hours	2 hours	
	⁰⁄₀	⁰⁄₀	⁰⁄₀	⁰⁄₀	⁰⁄₀	
Student No. 15	·126	·196	·142	·126	...	Sugar in urine *nil*
Student No. 16	·120	·206	·196	·167	·12	Do.

Both these cases were perfectly healthy students so far as we could make out from clinical observations.

Conclusions

(1) The average renal threshold for sugar in healthy Bengalee students is about ·175 per cent which is very nearly the same as in the case of Europeans.

(2) In rare cases, the renal threshold in healthy Bengalee students was somewhat high, as is also the case with healthy Europeans.

A THERAPEUTIC SALVARSAN DERI-VATIVE ALLIED TO SULPHARSENO-BENZENE AS PREPARED IN INDIA

It is for the first time in India that a salvarsan derivative of high therapeutic index has been prepared in the Brahmachari Research Institute, Calcutta. The compound is allied to sulpharsenol, myosalvarsan, sulpharsphenamine and other foreign sulpharsenobenzene preparations. Full reports of the toxicity results and curative value of this compound are under publication elsewhere.

ON THE THERAPEUTIC VALUE OF THIO-SARMINE IN THE TREAT- MENT OF SYPHILIS

The voluntary venereal hospital in Calcutta is the only government institution in India for the treatment exclusively of venereal diseases and only women are admitted here.

In this paper an attempt has been made to study the effect of thio-sarmine which is prepared in the Brahmachari Research Institute, Calcutta, for the treatment of syphilis.

The compound thio-sarmine is sulph-arseno-benzene or di-sodium-dioxy-diamino-arseno-benzene-methylene sulpho-nate, and is allied to sulfarsenol, kharsulphan, sulphars-phenamine, sulphostab, etc. It is a light yellow powder readily soluble in water. The solution used is made with distilled water freshly boiled before use and cooled. The solution is injected immediately after preparation. We have used 10 to 20 per cent solutions which are slowly injected either subcutaneously or intramuscularly into the deltoid or buttocks twice a week.

Generally we begin the treatment with a dose of 0·3 gramme and gradually increase the dose up to 0·6 gramme and the average total amount of 5·1 grammes was adminis-tered to each patient. During the treatment the intermediate doses were repeated, if found desirable.

The toxicities of thio-sarmine, sulfarsenol, sulpharsphena-mine are almost the same in experimental animals. Given intravenously the maximum tolerated dose of thio-sarmine = 300 milligrammes, majority tolerated dose = 360 milli-grammes, and the minimum lethal dose = 490 milligrammes per kilogramme of bodyweight, in the case of white rats.

Cases which gave positive Wassermann reaction and showed syphilitic manifestations, either local or general or both, at the commencement of treatment were treated with the drug. The blood of each case was examined for Wassermann reaction before, and two to three months after completion of treatment.

The therapeutic value of the drug was proved by the disappearance of syphilitic manifestations and by the Wassermann reaction remaining negative after completion of treatment.

The total number of cases treated by thio-sarmine was 30, of these 23 gave a strongly-positive Wassermann reaction and 7 a moderately-positive reaction. They were all cases of secondary syphilis.

Results of treatment.—The ulcers when present healed up quickly, condylomatous and elephantoid conditions disappeared gradually, adenitis subsided in the majority of cases and in those which had no suppuration eruptions over the body disappeared, and the general conditions markedly improved in all the cases. Reactions, such as diarrhœa, headache, gastritis, dermatitis were not observed in any of the cases during treatment. For effects on the Wassermann reaction see Table I.

We made a comparative study of the value of thio-sarmine in the treatment of syphilis by using the following drugs :—

      I.   Sulfarsenol.
     II.   Sulfarsenol and bismostab.
    III.   Novarsenobillon.
    IV.   Novarsenobillon and bismostab.
     V.   Novarsenobillon and mercurosol.
    VI.   Stabilarsan.
   VII.   Thio-bismol.
  VIII.   Quino-ido-bismuth.
    IX.   Bismuth-idol.

The symptoms of the cases treated by these drugs were more or less the same as in those treated with thio-sarmine and consisted of hard chancre, phagedenic sore, condylomata, elephantoid condition, adenitis, arthritis, secondary eruptions,* cauliflower-like growths, gummatous sore, iritis, keratitis, etc. A comparative statement of the effects of these . drugs on the Wassermann reaction is given in Tables II and III.

I. *Sulfarsenol.*—Total number of cases treated was 30; of these 19 gave a strongly-positive Wassermann reaction and 11 a moderately-positive reaction. Doses—12 centigrammes, 18 centigrammes and 24 centigrammes; two injections of each dose were given to each patient either intramuscularly or subcutaneously into the buttocks in 1½ months. In our experience, higher doses of sulfarsenol are not tolerated by the patients.

II. *Sulfarsenol and bismostab.*—Total number of cases treated was 10; of these 7 gave a strongly-positive Wassermann reaction and 3 a moderately-positive reaction. Doses of sulfarsenol—12 centigrammes, 18 centigrammes and 24 centigrammes; two injections of each dose were given intramuscularly or subcutaneously, and 6 injections of 0·5 cubic centimetre dose of bismostab were given intramuscularly to each patient alternately with the former in 2½ months. In our experience, higher doses of sulfarsenol and bismostab are not tolerated by the patients.

III. *Novarsenobillon.*—Total number of cases treated was 30; of these 27 gave a strongly-positive Wassermann reaction and 3 a moderately-positive reaction. Doses—0·3 gramme and 0·45 gramme; 4 injections of each dose were given intravenously to each patient in two months. In our experience, higher doses of novarsenobillon are not tolerated by the patients.

IV. *Novarsenobillon and bismostab.*—Total number of cases was 30; of these 28 gave a strongly-positive Wassermann reaction and 2 moderately-positive reaction. Doses of

novarsenobillon —0·3 gramme and 0˙45 gramme ; 4 injections of each dose were given intravenously and 8 injections of 0·5 cubic centimetre dose of bismostab were given intramuscularly to each patient, alternately with the former in $2\frac{1}{2}$ months. In our experience, higher doses of novarsenobillon and bismostab are not tolerated by the patients.

V. *Novarsenobillon and mercurosol.*—Total number of cases treated was 30; of these 27 gave a strongly-positive Wassermann reaction and 3 a moderately-positive reaction. Doses of novarsenobillon—0·3 gramme and 0·45 gramme; 4 injections of each dose were given intravenously and 8 injections of 1 cubic centimetre dose of mercurosol were given intramuscularly to each patient alternately with the former in $2\frac{1}{2}$ months. In our experience, higher doses of novarsenobillon and mercurosol are not tolerated by the patients.

VI. *Stabilarsan.*—Total number of cases treated was 30; of these 27 gave a strongly-positive Wassermann reaction and 3 a moderately-positive reaction. Doses—0·3 gramme and 0·45 gramme; 3 injections of each dose were given to each patient intravenously in $1\frac{1}{2}$ months. In our experience higher doses of stabilarsan are not tolerated by the patients.

VII. *Thio-bismol.*—Total number of cases treated was 15 ; of these 13 gave a strongly-positive Wassermann reaction and 2 a moderately-positive reaction. Dose—10 injections of 0·2 gramme were given intramuscularly into the buttocks to each patient in 2 months. In our experience, higher doses of thio-bismol are not tolerated by the patients.

VIII. *Quino-ido-bismuth.*—Total number of cases treated was 15 ; of these 13 gave a strongly-positive Wassermann reaction and 2 a moderately-positive reaction. Dose—6 injections of 4 cubic centimetres were given intramuscularly to each patient in $1\frac{1}{2}$ months. In our experience, higher doses of quino-ido-bismuth are not tolerated by the patients.

IX.   *Bismuth-idol.*—Total   number of cases treated was
10.   Of these 9 gave a   strongly-positive Wassermann reac-
tion and 1 a moderately-positive reaction.   Dose—8 injections
of 2 cubic centimetres were given intramuscularly to each
patient in 1½ months.   In our experience, higher doses of
bismuth-idol are not tolerated by the patients.

Observations

(1)   It will be seen from the above that thio-sarmine is the
most efficacious drug in the treatment of syphilis and its
manifestations.

(2)   Intolerance towards this drug is much less than with
other arsenobenzene compounds.

(3)   The effect on the Wassermann reaction is remarkable
in the case of thio-sarmine as will be seen from Tables II and
III ; the largest number of cases showing negative Wasser-
mann reaction after   treatment was noted in the case of thio-
sarmine.   •

In compiling this paper we have not in any way selected
cases for treatment as being likely to respond, but each case
has been taken in the order of admission, irrespective of the
gravity of disease or nature of symptoms.   All cases have
been positive in blood reaction and without exception the
lesions have been gross and extensive.   Side by side with
thio-sarmine treatment, other cases have been undergoing
treatment with other preparations.   Amongst the advantages
we have noted clinically are absence of reaction in cases
treated with   thio-sarmine.   The temperature hardly ever
rises, and there is practically no pain.   There have been no
nitritoid crises, nor anaphylactic conditions and hitherto no
arsenical dermatitis.   The drug may be regarded as one of
the most innocuous at present in use, its lack of toxicity
being most marked in comparison with its high efficacy.   It
appears to be, so far, most suitable both for hospital and

private use. Furthermore, the rapidity with which symptoms disappear compares very favourably with other preparations. Our experience in this hospital is that the ordinary Indian female patient is intolerant of novarsenobillon in doses over 0˙45 gramme and it is 'our practice never to exceed this. The usual onset of dermatitis, when it occurs, is after the 4th injection, or if the dose of 0˙45 gramme has been exceeded. With thio-sarmine the maximum dose has been up to 0˙6 gramme without the slightest untoward effect. It is to be remembered that the dosage of the arsenical compounds, such as novarsenobillon, neosalvarsan,· etc., has been based on the physical characteristics of the European and it is now known that the Indian female cannot tolerate a dose well-borne by her European sister. This does not, however, seem to apply in the case of thio-sarmine. We have observed no case in which the Indian female has shown intolerance even in the higher doses. It therefore appears clinically that it is of a comparatively low toxicity.

TABLE I

Table showing treatment with thio-sarmine intramuscularly and subcutaneously and its effect on Wassermann reaction in 30 cases

Case number	Doses in grammes			Total number of injections	Total quantity injected in grammes	Duration of treatment in months	Wassermann reaction	
	0·3	0·45	0·6				Before treatment	After treatment
1	1	2	7	10	5·4	2 months	Strongly positive	Strongly positive
2	2	4	6	12	6·0	2¼ ..	Do.	Do.
3	1	2	7	10	5·4	2 ..	Do.	Moderately positive
4	1	4	5	10	5·1	2¼ ..	Do.	Do.
5	1	4	6	11	5·7	2¼ ..	Do.	Do.
6	1	4	5	10	5·1	2 ..	Do.	Do.
7	1	4	5	10	5·1	2 ..	Do.	Doubtful
8	1	4	4	9	4·5	2 ..	Do.	Do.
9	1	2	3	6	3·0	1¼ ..	Do.	Do.
10	2	2	3	7	3·3	1½ ..	Do.	Negative
11	1	4	5	10	5·1	1½ ..	Do.	Do.

No.									
12	1	4	5	10	5·1	2½	..	Do.	Do.
13	2	4	..	6	2·4	1¾	..	Do.	Do.
14	1	4	5	10	5·1	1½	..	Do.	Do.
15	2	5	2	9	4·5	2	..	Do.	Do.
16	1	2	2	5	2·4	1½	..	Do.	Do.
17	1	2	2	5	2·4	1½	..	Do.	Do.
18	1	2	4	7	3·6	1¾	..	Do.	Do.
19	1	2	7	10	5·4	2	..	Do.	Do.
20	1	2	4	7	3·6	1¾	..	Do.	Do.
21	2	4	4	10	4·8	2	..	Do.	Do.
22	1	2	7	10	5·4	2	..	Do.	Do.
23	1	2	7	10	5·4	2	..	Do.	Do.
24	1	4	2	7	3·3	1 month	Moderately positive	Doubtful	
25	2	2	4	8	2·7	1	..	Do.	Do.
26	2	2	3	7	3·3	1	..	Do.	Do.
27	2	2	3	7	3·4	1	..	Do.	Do.
28	1	4	5	10	5·1	1½ months	Do.	Negative	
29	1	4	3	8	3·9	1¾	..	Do.	Do.
30	2	4	..	6	2·4	1 month	Do.	Do.	

TABLE II

Showing the comparative value of thio-sarmine and other drugs in figures on Wassermann reaction after treatment in 240 cases of syphilis

	Before treatment		Total number	After treatment			
	Strongly positive	Moderately positive		Strongly positive	Moderately positive	Doubtful	Negative
Thio-sarmine	23	7	50	2	4	8	16
Sulfarsenol	19	11	30	11	10	7	2
Sulfarsenol and bismostab	7	3	10	4	5	1	nil
Novarsenobillon	27	3	30	10	12	4	4
Novarsenobillon and bismostab	28	2	30	10	7	8	5
Novarsenobillon and mercuro-sol.	27	3	30	10	10	6	4
Stabilarsan	27	3	30	17	7	5	1
Thio-bismol	13	2	15	8	4	2	1
Quino-ido-bisruth	13	2	15	6	6	2	1
Bismuth-idol	9	1	10	4	3	nil	3

TABLE III

Showing the comparative value of thio-sarmine and other drugs, in percentage on the Wassermann reaction after treatment calculated from the figures in table II

	Strongly positive	Moderately positive	Doubtful	Negative
Thio-sarmine	6·6	13·3	26·6	53·3
Sulfarsenol	36·6	33·3	23·3	6·6
Sulfarsenol and bismostab	40·0	50·0	10·0	nil
Novarsenobillon	33·3	40·0	13·3	13·3
Novarsenobillon and bismostab	33·3	23·3	26·6	16·6
Novarsenobillon and mercurosol	33·3	33·3	20·0	13·3
Stabilarsan	56·6	23·3	16·6	3·3
Thio-bismol	53·3	26·6	13·3	6·6
Quino-ido-bismuth	40·0	40·0	13·3	6·6
Bismuth-idol	40·0	30·0	nil	30·0

OBSERVATIONS ON THE HÆMOLYTIC ACTION OF QUININE* AND ITS SALTS *IN VIVO* IN MAN AND ANIMALS

The present investigation was undertaken to determine whether quinine had any hæmolytic action *in vivo* on red corpuscles in man under healthy or diseased conditions—malarial or otherwise and in healthy animals, in other words, to determine whether hæmolysis is a constant feature *in vivo* after intravenous administration of quinine and its salts. For this purpose the authors have divided the cases into groups and the investigation carried out in the following way : Immediately before the injection, the red blood corpuscles of the peripheral blood of the individual were estimated by Bürker counting chamber and the hæmoglobin by Hellige's modification of Sahli's hæmoglobinometer and in each case the average of three estimations was taken. 3 to 5 grains of quinine bihydrochlor. dissolved in 10c.c. of normal saline were then injected intravenously, and the red blood corpuscles and hæmoglobin again estimated after $1\frac{1}{2}$ hours.

During this period patient was kept in bed and no food or water was given by the mouth. Observations were made at about the same hour of the day, *i.e.*, from 3 to 5 p.m.

In every case in a series of 5 cases of kala-azar and 5 of malaria it was observed that intravenous injection of quinine bihydrochlor. was followed by a distinct reduction of the red blood corpuscles and of hæmoglobin value of the peripheral blood. The cases were taken promiscuously from the wards.

THE ACTION OF QUININE ON A HÆMO-LYTIC SYSTEM *IN VITRO* AND ITS BEARING, IF ANY, ON THE MECHANISM OF BLACK-WATER FEVER

The present paper shows that solutions of quinine bihy-drochloride or hydrochloride in strengths not having any direct hæmolytic action on red corpuscles inhibit hæmolysis in a hæmolytic system. If, therefore, in the hæmolysis of black-water fever following administration of quinine, a hæmolytic system plays a part, then quinine hydrochloride or bihydrochloride would inhibit hæmolysis in that system, so far as experiments *in vitro* prove. Whether, at the same time, quinine helps in the development of a hæmolytic system in a susceptible individual, the authors are at present unable to say.

THE PREPARATION OF STABLE COLLOIDAL ANTIMONY

The use of colloidal metals in the treatment of bacterial diseases is getting more and more extensive and has given rise to many brilliant results. The remarkable trypanocidal properties possessed by antimony, its specific action against the leishmania, and the fact that in the colloids generally the ratio dosis curativa : dosis tolerata is very low, make it desirable to prepare a stable solution of colloidal antimony. Svedberg has obtained the isobutyl-alk-sol of colloidal antimony by a method similar to that of preparing colloidal arsenic. Svedberg's colloidal antimony is brownish-red by transmitted light and black by reflected light.[1]

There is mention of the preparation of this drug in the *Chemist and Druggist* (Vol. I, 1913), but so far as I am aware, none of the big manufacturing chemists in England are able to supply it. The reference in the *Chemist and Druggist* is probably to some colloidal compounds of antimony which have been prepared. Experiments conducted by Martindale on the chemical production of colloidal antimony have been unsuccessful.[2] Svedberg's colloidal antimony was very unstable and was precipitated within a short time after its production, and must, therefore, be useless for therapeutic purposes on a practical scale.

Our first experiments were performed with ethyl alcohol. It was found that no colloidal antimony could be

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[1] Ber. 38, 3615-3620, 1905, and Meggs' " Inorganic Chemistry."

[2] Extra Pharmacopœia, 1915, sixteenth edition.

obtained by passing sparks from an induction coil through coarse metallic antimony under ethyl alcohol. The method of passing a feeble current through this medium was tried but without success. The most successful medium was found to be chloroform, from which the colloid is obtained in fair concentration and from which solid colloidal antimony can be separated by evaporating the medium.

The details of the preparation are as follows : The apparatus consists of a fair-sized induction coil worked by an 8-volt accumulator. The electrodes, made of aluminium foil, are dipped in chloroform, into which are added coarse particles of pure metallic antimony freed from dust of antimony by sifting. On passing sparks through this medium some of the antimony passes into a powdery state and some goes into solution. On distilling off the solvent we obtain a tar-like substance, having a peculiar smell. The substance can be dried in a desiccator or in an air oven heated gently over the Bunsen burner, but apparently does not undergo much change, as it is freely soluble in chloroform after such treatment.

Colloidal solution of antimony is brownish-red by transmitted light and black by reflected light, being in this respect similar to Švedberg's colloid. It presents the Tyndall phenomenon. The therapeutic use of colloidal metallic antimony has already been described in the Indian Medical Gazette, May, 1916. Further observations on the use of this drug in the same disease have shown similar beneficial results.

The author's grateful thanks are due to Dr. Rasik Lal Datta who helped him in the prepartion of colloidal antimony.

Editor's Note

Colloidal antimony is not so stable as was originally considered by the author.

ANTIMONY TREATMENT OF KALA-AZAR

An article under this title by Sir Leonard Rogers appeared in *Nature* of December 16, 1939, p. 1003, which presented in very bare outline the history of the use in India of anti-mony compounds to combat kala-azar. Sir Upendranath Brahmachari, whose work in connexion with the introduction and use of urea stibamine was referred to briefly, has sub-mitted to *Nature* a long statement surveying in some detail the work which led up to the preparation of this substance and reporting on the results obtained. He states that, contrary to Sir Leonard Rogers' statement, urea stibamine was not patented, and claims that divergent results obtained by different investigators were due to the fact that various manufacturers put on the market so-called urea stibamine which did not conform to his specification. Reference is also made to the cost of treatment with antimony compounds. Sir Leonard Rogers stated in his article that a course of treatment with urea stibamine cost £3 in 1925. In the intervening years, this has happily been reduced; Sir Upendranath states that urea stibamine is now supplied by the Government at Re. 1 per gram, and since 1·5 gm. is sufficient for complete cure, the total cost of the drug to-day is now Rs. 1-8 (about 2*s*. 3*d*.). This is a reduction on which all who have been concerned are to be congratulated.

CHEMISTRY OF UREA STIBAMINE

In his article on the '' Antimony Treatment of Kala-azar,'' [1] Sir Leonard Rogers referred to the findings of an early worker on the constitution of urea stibamine ; reference was not made to later work on this subject, notably that of Gray *et al.*[2]

To some extent the controversy about the constitution of urea stibamine is comparable to that of atoxyl when it was discovered by Ehrlich. Gray *et al.* have noted that the most interesting of the more important derivatives of *p*-amino-phenyl stibinic acid is a material prepared by Brahmachari (1922) under the name ' urea stibamine ' by heating *p*-amino-phenyl stibinic acid with urea solution. They have shown that the ' essential active principle ' in urea stibamine is s-diphenyl-carbamide-4 : 4′ distibinic acid

$$O \overset{HO}{\underset{HO}{=\!\!=}} Sb.C_6H_4.NH.CO.NH.C_6H_4.Sb \overset{OH}{\underset{OH}{=\!\!=}} O,$$

which is rendered water-soluble in the presence of protective colloids, and that this active principle is responsible for the remarkable therapeutic properties of urea stibamine. So far as I am aware, these findings have not been contradicted by any subsequent observer.

I would also point out that Gray *et al.* found fairly constant results from analysis of urea stibamine (Brahmachari),

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[1] *Nature*, **144**, 1103 (1939).

[2] *Proc. Roy. Soc.*, B. **108**, 54 (1931).

as shown in the accompanying table (the discrepancy in the
antimony content of samples examined by them being due
to varying amounts of the protective colloids present).

Carbon	Hydrogen	Nitrogen	Antimony
—	—	6·75	44·19
—	—	6·77	44·49
20·2	3·0	—	—
20·9	2·9	—	—
20·5	2·8	—	—
20·9	3·0	—	46·4
21·53	2·67	—	—
21·16	2·8	—	46·8
20·17	2·91	6·47	48·6

As already noted in *Nature*, [1] the divergent results obtained
by different investigators were due to the fact that various
manufacturers put on the market so-called urea stibamine
which did not conform to my specification. This no doubt
led to the conclusion of early workers that the so-called
urea stibamine varied widely in its antimony content and
was uncertain in its composition.

[1] *Nature*, **145**, 546 (1940).

NOTE ON THE HISTORY OF THE TREATMENT OF KALA-AZAR WITH UREA-STIBAMINE

History of the introduction of inorganic antimonials in the treatment of Kala-azar

Before the introduction of antimonial treatment, various methods of treatment of kala-azar had been tried without success. They need not be described here.

It was not until attention was directed to antimony preparations that a specific treatment was discovered. The therapeutic properties of antimony were quite well known even in the 15th century and up to the 17th century preparations of antimony were supposed to be remedies of various afflictions. Cups of metallic antimony – 'Cups claiming to be panaceas for all diseases '—were in considerable vogue. Wine left in these cups for sometime gave rise to some soluble antimonial compounds in the cups and through improper use of such cups large number of monks were at times found to be afflicted with some mysterious diseases and some even died. This gave rise to the name *Antimonk* whence the metal (Latin name *stibium*) came to be known as antimony. Later the Faculty of Physicians of Paris banned the use of antimonial remedies and the ban continued for a long time. Similarly the graduates in medicine in the University of Heidelburg had to swear never to use antimony.

Manson was the first to advise antimonial preparations in the treatment of kala-azar. In 1913 Vianna in Brazil reported the cure of the South American forms of cutaneous and mucous leishmaniasis with tartar emetic' administered intravenously. Di Cristina and Caronia (1915) in Sicily were the first to record the successful use, of tartar emetic intravenously in Mediterranean kala-azar. About the same year Rogers claimed to have obtained favourable results in a number of cases in India with tartar emetic independently of Di Cristina and Caronia. Later, towards the fall of the same year sodium antimonyl tartrate was introduced by Brahmachari. It was found to be less toxic than tartar emetic and it replaced the latter in the hands of many observers.

Experience showed that both tartar emetic as well as sodium antimonyl tartrate were not free from many disadvantages. Thus Murison (1927), Director of Public Health, Assam, wrote as follows regarding the disadvantages in the use of potassium and sodium antimony tartrates :—

"The treatment of the disease in Assam with tartar emetic began in 1919, when only a comparatively small number of cases were treated. It was soon realised that this drug was not without its dangers and it was soon replaced by sodium antimonyl tartrate, which was found much safer and gave much more satisfactory results."

" Although treatment with sodium antimonyl tartrate has been very successful, it has the disadvantage of being long and tedious. Treatment is, therefore, difficult to enforce, as patients who have been completely incapacitated by the disease, improve so considerably after a few injections that they discontinue treatment altogether or attend very irregularly. This irregularity makes it very difficult to effect complete cures. Inspite of the regulation in force under the Epidemic Diseases Act to compel petients to undergo a complete

course of treatment, the campaign against kala-azar in Assam was greatly handicapped by the larger number of patients who are stopping treatment."

" It was felt that the above difficulties would be still further overcome if some drug could be introduced which was not only as efficacious as sodium antimonyl tartrate but took a much shorter time to effect a cure."

Rogers (1928) noted that solutions of potassium and sodium antimonyl tartrates "do not keep well especially in a tropical climate, and the salts are readily decomposed, especially by bacterial contamination when very toxic substances appear to be produced and form a fine precipitate." Further he " recorded accidents resulting from solutions sterilized in the autoclave in rubber-capped flasks becoming contaminated in the hot, humid, rainy season in Calcutta, through repeated punctures of rubber caps in taking up the dose. Very serious toxic symptoms appeared within a few hours, and even terminated fatally." Napier (1927) of the Calcutta School of Tropical Medicine noted that among other complications due to antimonyl tartrate injections may be mentioned coughing, vomiting, pneumonia and lung complications, aggravation of kidney and bowel complications, joint pains, eruptions, very marked slowing of the heart and very sharp reactionary rise of temperature.

All these observations showed that though the efficacy of tartar emetic and sodium antimonyl tartrate against kala-azar was undoubted yet patient chemo-therapeutical researches were very necessary for the discovery of an antimonial which would not produce the bad effects noted above.

History of the introduction of urea stibamine in the treatment of kala-azar

An extensive series of chemical and therapeutical experiments with various antimonials were carried out by

Brahmachari between the years 1915-1921, at the Hospital attached to the Campbell Medical School, Calcutta. In 1919, Brahmachari was helped to carry out his chemotherapeutical researches by a grant from the Indian Research Fund Association. At first several new inorganic antimonials were synthesized and tried. If a record of the various antimonials tried by Brahmachari were kept then, following the German practice, urea stibamine could have been described by a figure of three digits. The curious reader may find an account of some of Brahmachari's experiments on various antimonials in *Brahmachari's Kala-azar* (Butterworth & Co., Calcutta, 1917 and 1926).

A certain amount of success was obtained from the use of metallic antimony in a state of fine subdivision (1915) and colloidal metallic antimony (1916). It has been subsequently observed by Brahmachari and co-workers (1930) that when metallic antimony was injected intravenously in a state of fine subdivision in experimental animals, its particles were picked up by the same cells in the spleen as those that harboured the parasite of kala-azar and that the struggle that ensued in the fight ended most remarkably in the complete destruction of the parasites in the speediest way. The advantage of intravenous injection of metallic antimony in a state of fine subdivision was that the number of injections generally required to bring about complete cure was not more than three or four. The chief objection to its use was the complicated technique of the operation of injection which was a serious obstacle in the mass treatment of the disease. The use of colloidal metallic antimony was subsequently discontinued, as experience showed that it was not so stable as originally considered. Further, the technique of its preparation was somewhat complicated and it was difficult to prepare on a large scale and the number of injections required to effect a cure was large.
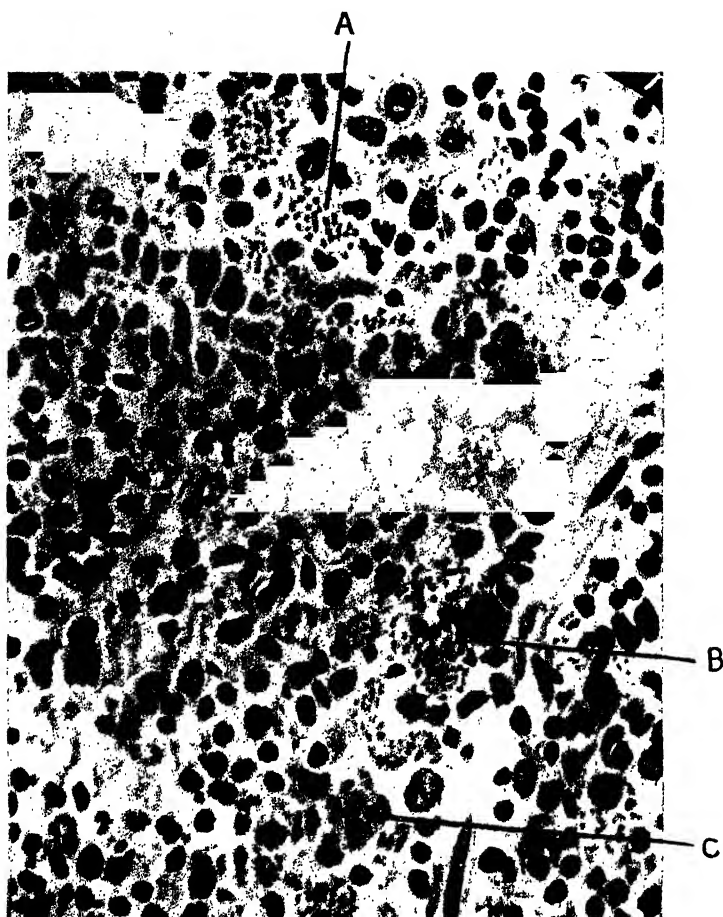
It may be mentioned here that colloidal metallic anti-
mony and metallic antimony in a state of fine subdivision
were not available in India when work was begun by
Brahmachari on the treatment of kala-azar with antimonials.
A new method for the preparation of colloidal metallic
antimony was subsequently evolved by him (1916) by
using a new technique which need not be described here.
It was found to be more stable than Svedberg's colloidal
antimony. Metallic antimony in a state of fine subdivision
was prepared by him following the method of Plimmer
(1911).

To avoid the disadvantages of the inorganic antimonials Brahmachari subsequently turned his attention to the study of organic antimonials. In this work which was carried out between 1915 and 1921, he was inspired by the idea that an antimonial having a constitution similar to that of atoxyl,
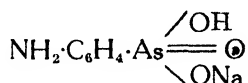
$$NH_2 \cdot C_6H_4 \cdot As \underset{\diagdown ONa}{\overset{\diagup OH}{=\!=\!=}} O$$

which was found by Ehrlich to be effective in the treatment of sleeping sickness might prove useful in the treatment of kala-azar.

Towards the fall of 1919, preliminary observations on the successful preparation of p-stibanilic acid

$$NH_2 \cdot C_6H_4 \cdot Sb \underset{\diagdown OH}{\overset{\diagup OH}{=\!=\!=}} O$$
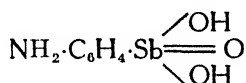
and its salts of the type

$$NH_2 \cdot C_6H_4 \cdot Sb \underset{\diagdown OR}{\overset{\diagup OH}{=\!=\!=}} O$$
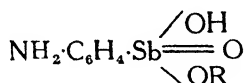
were communicated by Brahmachari to the Indian Research Fund Association. The results were so encouraging that it was asserted by him that the " manufacture of this compound in India would be as important for the treatment of kala-azar as cinchona plantation for the treatment of malaria.

In addition to the defects of the antimonials hitherto known, it was observed by Brahmachari that most of them were very painful when administered intramuscularly. Hence his attention was drawn to the synthesis of an antimonial allied to atoxyl which would prove painless when administered intramuscularly. After a large number of experiments, Brahmachari thought that p-amino-phenyl stibinic acid if it could be combined with urea might prove painless, as urea when administered with certain drugs conferred anæsthetic properties on them, e.g., quinine urea.

This was the genesis of the discovery of urea stibamine, which was first prepared in 1920.

Constitution of urea stibamine

Controversy about the constitution of urea stibamine is comparable to that of atoxyl when the latter was discovered by Ehrlich. The situation is well summarised by Gray *et al* (1931) who studied the constitution of urea stibamine. They noted that " the most interesting of the more important derivatives of p-amino-phenyl stibinic acid is a material prepared by heating stibanilic acid with urea solution, introduced by Brahmachari (1922) under the name of ' urea stibamine,' the nature of which has been the subject of conflicting opinions by other workers as well as by Brahmachari himself." Fairly constant results from analysis of ur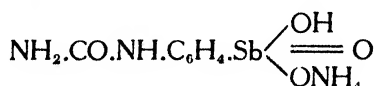ea stibamine as prepared in the Brahmachari Research Institute have been shown in the paper of Gray *et al* (the slight discrepancy in the antimony content of samples examined by them being due to varying amounts of the protective colloids present).

It was at first thought by the present writer (1932) that urea stibamine had the constitution,—

$$NH_2.C_6H_4.Sb\diagup\!\!\!\!\diagdown\begin{matrix}OH\\OH\end{matrix}\;\;O\cdot CO(NH_2)_2$$
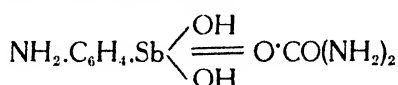
In a later investigation (1924) this was modified by him to

$$NH_2.CO.NH.C_6H_4.Sb\diagup\!\!\!\!\diagdown\begin{matrix}OH\\ONH_4\end{matrix}\;\;O$$

which was confirmed by Niyogi (1928).

According to Gray *et. al*, the essential active substance of ' urea stibamine (Brahmachari)' is

$$O\Longrightarrow\diagup\!\!\!\!\diagdown\begin{matrix}HO\\HO\end{matrix}Sb.C_6H_4.NH.CO.NH.C_6H_4.Sb\diagup\!\!\!\!\diagdown\begin{matrix}OH\\OH\end{matrix}\Longrightarrow O$$

(s-diphenyl-carbamide-4 : 4'-distibinic acid), rendered water-soluble.

Certain observers in Calcutta who have analysed certain brands put up in the market of Calcutta under the name of urea stibamine have held the view that it varied widely in its antimony content and was uncertain in its composition. This view is unjustified and misleading and has created a mistaken impression in the minds of subsequent authors who have quoted them. It is partly due to the fact that some improperly prepared brands have been put up on the market by untrained persons under the name of urea stibamine. It is unfortunate that the writers referred to above while publishing their results never took the precautions of mentioning the manufacturers' names and who knows whether the compound tested by them was anything but that antimony compound which was discovered by Brahmachari under the name of urea stibamine. It is also evident that the above authors missed the active principle of urea stibamine (Brahmachari) described subsequently by Gray *et al.* This active principle is perhaps the " antimony in some form " present, according to the writers referred to above, in urea stibamine but whose constitution they were not able to determine.

Therapeutic value of urea stibamine

Shortly after the discovery of urea stibamine in 1920, the drug was administered by Brahmachari on patients in the Campbell Medical Hospital. The results were beyond expectation, and this encouraged administration of the medicine on a mass scale. Actual records of a large number of cases in the wards of the Calcutta Medical College Hospitals under different physicians in 1923 showed that the disease was radically cured in three weeks after injection of 1.5 gms. of urea stibamine.

The most exhaustive observations were made by Major Shortt (1923), of the Indian Medical Service, Special Kala-azar

Officer, Pasteur Institute, Shillong, and at present Director, Pasteur Institute, Guindy, and Dr. R. Sen. The drug was sent to Shortt for trial at the instance of the Indian Research Fund Association.

In their Final Report on the use of urea stibamine in kala-azar, they noted :—

" We consider that the value of urea stibamine has been established as the most efficient drug at present in use for the treatment of the Indian kala-azar. The conclusion is based not only on a series of cases of which we have published the details, but in addition on experience gained in many other cases, both Indian and European, which have passed through our hands or which have been treated with urea stibamine under our direction, a number totalling nearly one hundred cases."

Later on Shortt as Director, Kala-azar Commission (1932), stated :—

" We found urea stibamine an eminently safe and reliable drug and, in seven years we treated some thousands of cases of kala-azar and saw thousands more treated in treatment centres. The acute fulminating type characteristic of the peak period of an epidemic responds to treatment extraordinarily promptly, and with an almost dramatic cessation of fever, diminution in size of the spleen and return to normal condition of health. It may be expected that similar beneficial results will be obtained in other epidemics of the disease."

These observations were confirmed by a large number of medical men both Indian and European, employed in the Assam Tea Plantations, and in Government Hospitals in Bengal and other places. The use of the drug was not confined to India but is now used with success in Greece, France and China.

It is not within the scope of the present paper to give an account of the use of other antimonials in the treatment of kala-azar, notable amongst which is neo-stibosan which has been used with success in the treatment of kala-azar by Napier and others nor is it intended to give a detailed account of their relative value. Among other antimonial compounds used by the present writer may be mentioned' sodium N-phenyl-glycine-amide-4-stibinate (antimony analogue of tryparsamide) and sodium-sulpho-methylene-stibanilate. I shall content myself by giving here a brief summary of the results obtained by the Government of Assam about the comparative value of urea stibamine and neo-stibosan and that of a few observers outside India.

Extracts from the Annual Reports of the Government of Assam

The use of urea stibamine on an experimental scale was started by the Assam Government from 1925. ,The results were so encouraging that it began to be used on a mass-scale from 1928.

1932

" Experiments with neo-stibosan were continued side by side with urea stibamine. *The consensus of the medical opinions received after submission of their report under review appears to be in favour of urea stibamine in regard to treatment of persons in rural areas where there is no indoor accommodation for patients at the treatment centres* and where it is inconvenient for the patients to visit the centres daily."

1933

" Urea stibamine was our main-stay in the treatment of kala-azar. *The treatment of kala-azar with neo-stibosan,* which was extended to indoor patients and to such *outdoor*

patients as voluntarily accepted it, was stopped during the latter part of the year. Neo-stibosan was given a trial in the intensive treatment of kala-azar. In rural areas the results were not encouraging. Its administration was, therefore, restricted to urban areas only where hospital conditions exist."

1934

" The administration of neo-stibosan is restricted to urban areas only where hospital conditions exist."

1935

"The treatment of kala-azar with neo-stibosan was stopped in this province during the year under review."

1936

" The treatment throughout the province is by means of intravenous injection with urea stibamine."

The comparative merits of urea stibamine and neo-stibosan tested in China (Lee and Chung, 1935), Greece (Lorando, 1937, Kaminopetros, 1938), France (Oelsnitz, 1934), have shown the former to be superior. It will then be seen from the above that the claims of certain writers that neosti-bosan is superior to urea stibamine are not warranted. To-day urea stibamine stands pre-eminent in the treatment of kala-azar all over India wherever the disease occurs and it has held its position for over 20 years.

The Director of Public Health, Assam, in his Annual Report for 1933, summarized the saving of lives by the use of urea stibamine as follows :—

" Urea stibamine was our main-stay in the treatment of kala-azar. Since 1933, when reliable figures for the disease first became available to the end of the year under report no less than 328, 591 persons have been brought under treatment. It is no exaggeration to say that approximately

3·25 lakhs of valuable lives have been saved to the province.''

Comparative statement of the number of cases of kala-azar treated and deaths in the province of Assam from 1925 to 1936.

ASSAM. 1925-1936
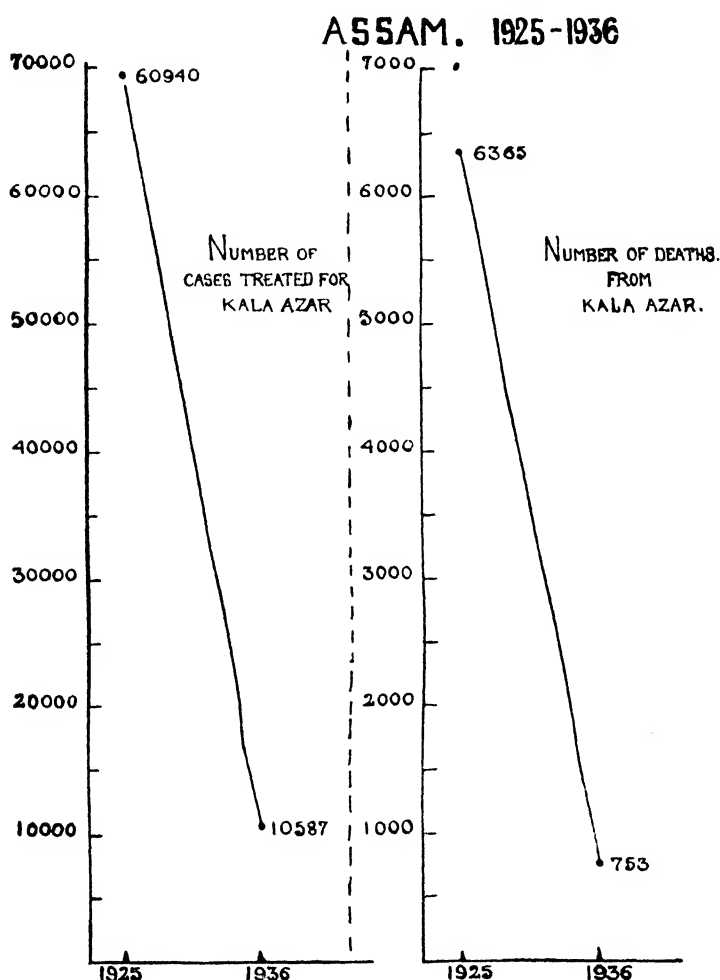
The timely discovery of the drug has prevented the spread of the disease in Assam and Bengal, and thus probably saved these places from the horrors witnessed earlier in Assam (1890-1925). Urea stibamine would have probably been just as effective in the cure of the Burdwan fever

which raged over Western and Central Bengal between 1854 and 1874, were it available at that time and if it was an epidemic of kala-azar as Rogers thought. According to the Indian Medical Gazette (1873) this Burdwan fever converted some parts of the districts of Burdwan and Hooghly into " a valley of the shadow of death" into which if any human being, whether robust or weak, well-nourished or the opposite, entered, he was certain to get a very severe attack, and he might consider himself very fortunate if he escaped out of it with life.

Cost of Urea Stibamine

To-day urea stibamine is supplied to the Government at the rate of Re. 1/- per gramme. Calculating that the amount of urea stibamine required for a complete cure is 1·5 grammes, which is frequently much less, the total cost of the drug required is Rs. 1/8/- or less. The cost of dietary of a patient during his stay in hospital for three weeks at the most, is Rs. 10/8/-. Total cost = Rs. 12/-.

Calculating that the amount of sodium antimonyl tartrate required for a complete cure is 5 grammes and not infrequently more, the cost of medicine is about Rs. -/2/6, or more. The cost of dietary of a patient during his stay in hospital for 3 months at least, is Rs. 45/-. Total cost = Rs. 45/2/6, which is little less than *four times* the cost required in the case of urea stibamine. These figures show that the cost of treatment of kala-azar even in a poor country like India with aromatic antimonials compares very favourably with that of malaria with quinine. In private cases, the cost will be much greater in the case of tartar emetic, if one takes the doctor's fees into consideration. It is only in charitable dispensaries that the cost is likely to be less in case of tartar emetic, but the time to be spent in the case of tartar emetic is much greater than in the case of urea

stibamine, and in the case of a poor man who has to earn his own food, the time element is of most importance, and the sooner he can get cured the better it is for himself and his family, if he is the earning member. The short course of treatment which is most desirable in the interests of the sick as well as of the man-power of the labourers in the affected areas who constitute the largest number of victims, specially in the tea-growing province of Assam, together with the great cheapness of the cost of treatment by means of organic antimonials in the present day, has led to the exclusive adoption of urea stibamine for the treatment of kala-azar in Assam.

References

The bibliography is not intended to be complete and mostly refers to some of the facts mentioned in the paper :

Brahmachari	...	1911	Indian Medical Gazette, 46, pp. 340-43, Sept., No. 9.
		1915	Indian Medical Gazette, 50, 455.
		1915	Indian Medical Gazette, Vol. 50, No. 12, 455-57.
		1916	Lancet, Oct., 21.
		1916	Indian Medical Gazette, 51, 173.
		1917	Kala-azar and its Treatment (1st edition).
		1920	Kala-azar and its Treatment (2nd edition).
		1922	Indian Journal of Medical Research, 10, 508.
		1928	A Treatise on Kala-azar, John Bale Sons & Danielson, Ltd.
		1930	Trans. Royal Society of Tropical Medicine and Hygiene, 23, 623.
Chopra	...	1936	Tropical Therapeutics.
Di Cristina and Caronia	...	1915	Bull. Soc. Path. Expt., 1915 Feb., VIII, Vol. No. 2, p. 63.
			Sulla terapia della Leishmaniosi interna. La Pediatric. Bd. XXIII. No. 2.
d'Oelsnitz and Carcopino	...	1934	Tropical Diseases Bulletin, 31, 232.
Donovan, C.	...		British Medical Journal, July 11, p. 79.
			Lancet, July 11, p. 44.
Foster	...	1924	Indian Medical Gazette, 59, 391
Gray et al	...	1931	Proceedings, Royal Society, 108, 54.
Ghosh, Chopra & Chatterjee	...	1928	Indian Journal of Medical Research, 16, 461.
Greig & Kundu	...	1925	Indian Journal of Medical Research, 12, 679.

Karpinopetros   ... 1938 Hippocrate (Athens), Fascicule No. 4.
Kapur   .. 1925 Indian Medical Gazette, 60, 206.
Lee and Chung   ... 1935 Chinese Medical Journal, 49, 1281.
Leishman, W. B ... 1903 British Medical Journal, May 30, 1252-54 and Nov. 21.
                                                                    1376-77.
Lorando   ... 1937 Bulletins et Memoires de la societe medicale des
                                        Hopitaux de Paris, No. 8.
Mackie   ... 1921 (Correspondence)
                           Indian Medical Gazette, Feb., Vol. 56, No. 2, pp. 76-77.
Michael   ... 1925 A Treatise on Kala-azar by Brahmachari, 1928,
                                                                    p. 130.
Murison   ... (1927-1931) Annual Public Health Report of the Province
                                        of Assam for the years 1926-1930.
                     1927 Annual Public Health Report of the Province of
                           Assam for the year 1925.
                           Indian Medical Gazette, January, pp. 52-54.
                     1927 Health Bulletin No. 9 (Government of India Central
                           Publication Branch) Calcutta, 12-14.
                     1932-37 Annual Public Health Reports of the Province of
                           Assam for the years 1931-1936.
Napier   ... 1923 " Stibosan " (Von Heydon " 471 ").
                     1925 Indian Medical Gazette, Oct. Vol. 60, No. 10, pp, 466-
                                                                    67.
                     1923 Indian Medical Gazette, 58, No. 12.
                 ,   1927 Kala-azar, pp. 158-59.
                     1927 Indian Medical Gazette, Vol. 61, No. 11, p. 753.
Niyogi   ... 1928 Journal of Indian Chemical Society, 5, 753.
                     1940 Nature, 368, June 29, 1940, pp. 1021-22.
Plimmer   ... 1911 Pharmaceutical Journal, June 10, p 793.
Price   ... 1924 Indian Medical Gazette, 59, 464.
Rogers   ... 1897 Indian Medical Gazette, Vol. 32. pp. 401-64.
                     1904 Indian Medical Gazette, Vol. 39, p. 158 and British
                           Medical Journal, Vol. I., p. 1249.
                     1908 Fevers in the Tropics.
                     1915 British Medical Journal, 2, p. 197.
                     1928 Recent Advances in Tropical Medicine, p. 21.
                     1939 Nature, No. 3659, Dec. 16, pp. 1003-4.
Shortt & Sen   ... 1923 Indian Journal of Medical Research, Vol. 12, No. 2,
                           pp. 335-38.
                     1923 Indian Journal of Medical Research, 11, p. 653,
                     1924 Indian Journal of Medical Research, 12, p. 335.
Shortt   ... 1932 Indian Medical Research Memoirs, Memoir. No. 25,
                                                                    p. 3.
Vianna and
  Machado   ... 1913 Bol. da. soc. Brazil, de Dermatologia, 1913, II.
                           French Resume, 34, pp. 40-41.
                           Tropic. Dis. Bull., Feb., 1914, Vol. 3, p. 14.

EDITOR'S NOTE

Brahmachari's present paper will help the reader in finding out the gaps and inaccuracies in Rogers' article on *The antimony treatment of kala-azar* published in Nature, December 16, 1939, which is likely to give rise to comments about the pioneers who have worked in this line since the discovery of the successful treatment of the disease by the use of tartar emetic. In this article Rogers reiterates his claims to priority for originating and using tartar emetic successfully in kala-azar which he had already made in his Correspondence, British Medical Journal, July, 1915, but which was repudiated by the Editor, Tropical Diseases Bulletin, August, 1915, who remarked that it is to Di Cristina and Caronia must be given the priority. The gaps and inaccuracies have been pointed out in Nature, News and Views, April 6, 1940, and more fully set forth in the present paper. In an article in Science and Culture, March, 1941, *On the Conquest of Kala-azar*, written by the Editor of the Journal, there is also a fairly detailed account of the researches conducted by Brahmachari and how he was subsequently led to the discovery of urea stibamine. To avoid the disadvantages of the antimonyl tartrates in the treatment of kala-azar, Brahmachari turned his attention to the study of aromatic antimonials. In this work he was inspired by the idea that an antimonial having a constitution allied to that of atoxyl which was found by Ehrlich to be effective in the treatment of sleeping sickness, might prove useful in the treatment of kala-azar. Towards the fall of 1919, when he was just financed for his researches by the Indian Research Fund Association, p-stibanilic acid was synthesized for the first time in India in his laboratory. His researches under the auspices of the Indian Research Fund Association with aromatic antimonials resulted in the synthesis of many new antimony compounds including derivatives of p-stibanilic acid and culminated in the discovery of urea stibamine. They are to be found in a series of papers in the Indian Journal of Medical Research entitled *Chemotherapy of Antimonial Compounds in Kala-azar Infection* but have unfortunately found no reference in Rogers' article. In Nature, June 29, 1940, a letter from Brahmachari on the chemistry of urea stibamine indicated the error in Rogers' statement about its constitution. Rogers has not stated that sodium antimonyl

tartrate was first introduced by Brahmachari in the treatment of kala-azar. He has made no reference to the work of Castellani who used tartar emetic almost simultaneously with him, of Shortt who as early as the period when he was Director of Pasteur Institute, Shillong, reported of having obtained most brilliant results in the treatment of kala-azar by the use of urea stibamine, of the Kala-azar Commission, India, who used exclusively this drug during the whole course of its existence for seven years and who treated thousands of cases, and of Napier who tried stibosan and neostam in the early days of antimonial treatment. No reference has been made to the various antimonials which have been used intermuscularly in the treatment of kala-azar, such as, a special brand of sodium antimonyl tartrate used by Napier, Castellani's solution of tartar emetic, hyperacid antimonyl tartrate, antimony analogue of tryparsamide and sulpho-methyl stibanilate, the latter three having been used by Brahmachari. Rogers' figures about the cost of treatment of kala-azar with urea stibamine are incorrect. Rogers has made no reference to the intensive treatment of kala-azar which was introduced by Brahmachari and gave good results in the hands of Brahmachari, Napier and others.

It is evident that Rogers' article though intended to present a " very bare" outline of the history of the use in India of the antimony compounds to combat kala-azar," as Nature puts it, is much too one-sided. His attempt to prove that neo-stibosan, a German preparation, is superior to urea stibamine is not borne out by the experience of the Government of Assam extending over several years and in many places outside India It may be mentioned here *en passant* that in the British Encyclopædia of Medical Practice, Surveys and Abstracts, 1939, Rogers has not even mentioned the name of urea stibamine in the present day treatment of kala-azar in India, though it is well known that this drug is now in universal use throughout India both by Government as well as by the public; and, as has been recently pointed out by the Editor of the Indian Medical Gazette, ' it is known even to the man in the street.' Rogers has not stated in his article that the German preparations, such as, neo-stibosan, stibosan and solustibosan were patented drugs.

From what have been shown in Brahmachari's paper, it appears that Rogers has failed to give a true picture of the most popular treatment of kala-azar in India in the present day, his

reference to the work of the pioneers in the early days of antimony treatment of kala-azar is meagre and his ideas about the cost of treatment with urea stibamine require revision and one is constrained to conclude that his   article   should be   revised and rewritten if it has to be of any value from a historical standpoint.

THE TREATMENT OF KALA-AZAR BY THE INTRAMUSCULAR INJECTION OF A NEW ANTIMONY COMPOUND—NEOSTIBENE

Since the discovery of antimony as a specific for the treatment of kala-azar attempts have been made from time to time to discover antimonials which could be administered intra-muscularly. The following may be mentioned out of such antimony compounds :

(1) Tartar emetic.
(2) Hyper-acid antimonyl tartrate with urethane.
(3) Sodium N-phenyl-glycine-amide-4-stibinate of sodium (antimony analogue of typarsamide).
(4) Sodium sulpho-methyl-stibanilate.
(5) Neo-stibosan.
(6) Neo-stibene.
(7) Antimony oxide dissolved in glycerine.

This paper gives a series of cases of kala-azar treated with intramuscular injection of neostibene. This compound is a derivative of *p*-amino-phenyl stibinic acid in organic combination. It is an amorphous greyish white powder and fairly soluble in water. Its antimony content is nearly 41%.

Neo-stibene has been prepared in the Brahmachari Research Institute under the direction of the writer.

Case I

Anath Nath Mitra, Hindu male, æt. 17, was admitted into the Tropical Ward of the Carmichael Medical College Hospital on 16-5-41 with the following complaints : (1) Fever (with double rise of temperature)—1 year. (2) Epistaxis

and bleeding from gums—1 year. (3) Spleen and liver extended 4″ and 1½″ respectively below the costal margin.

Blood examination report on admission on 16-5-41 : Hb— 45%, R. B. C.—2.2 mill., W. B. C.—3,437, Poly—60%, Lympho—35%, Mono—1%, Eosino— 4%, M.P. – nil. Aldehyde test—positive. Spleen puncture—L. D. bodies found.

Treatment : Neostibene was given.intramuscularly twice a week. Total amount injected was 3 gms.

Results of treatment on 12-7-41 : (1) The patient became afebrile after 8 injections of 0.1 gm. (2) Epistaxis stopped. (3) Spleen − 1½″ and liver—1″ below costal margin. (4) Blood report on 12-7-41 : Hb—65%, R. B. C.—3.8 mill., W. B. C.—4,737, Poly—68%, Lympho 28%, Mono—2%, Eosino—2%, No L. D. bodies found on spleen puncture.

Case II

Hrishikesh Dhar, Hindu male, æt. 31, was admitted into the Tropical Ward of the Carmichael Medical College Hospital on 12-5-41 with the following complaints : (1) Irregular intermittent fever—1 year. (2) Pain in the region of liver and spleen—6 months. (3) Spleen and liver extended 3½″ and 2″ respectively below the costal margin. (4) Diarrhœa—6 months.

Blood examination report on admission on 12-5-41 : Hb— 40%, R. B. C.—2.1 mill., W. B. C.—2,500, Poly—55%, Lympho—45%, Mono—nil, Eosino—nil. M. P.—nil. Aldehyde test—positive. Spleen puncture—L. D. bodies found.

Treatment : Neostibene was injected intramuscularly twice a week. Total dose injected was 3.5 gms.

Results of treatment on 10-7-41 : (1) The patient became afebrile after 8 injections of 0.1 gm. (2) Liver—not palpable, spleen—1½″ below the costal margin. (3) Blood report on 10-7-41 : Hb—60%, R. B. C.—3·2 mill., W. B. C.—5,000, Poly—65%, Lympho—33%, Mono—nil, Eosino—2%. No L D. bodies found on spleen puncture.

Case III

Maharam Ali, Mahomedan male, æt. 18, was admitted into the Tropical Ward of the Carmichael Medical College Hospital on 25-5-41 with the following complaints : (1) Irregular intermittent fever—1 year. (2) Spleen and liver extended 3″ and 1″ respectively below the costal margin. (3) General weakness and emaciation.

Blood examination report on admission on 23-5-41 : Hb—50% , R. B. C.—2·5 mill., W. B. C.—3,125, Poly—60%, Lympho—40%, Mono—nil, Eosino— nil. M. P.—nil. Aldehyde test—positive. Spleen puncture—L. D. bodies found.

Treatment : Neostibene was given intramuscularly twice a week. Total dose injected was 3 gms.

Results of treatment on 10-7-41 : (1) The patient became afebrile after 8 injections of 0.1 gm. (2) Liver—not palpable, spleen—1″ below the costal margin. (3) Blood report on 10-7-41 : Hb—60% , R. B. C.—3.2 mill., W. B. C.—5,000, Poly—66%, Lympho—30%, Mono—2%, Eosino—2%. No L. D. bodies found on spleen puncture.

Case IV

Dwijendra Lal Roy, Hindu male, æt. 37, was admitted into the Tropical Ward of the Carmichael Medical College Hospital with the following complaints : (1) Irregular intermittent fever—2 years. (2) Spleen—3″ below the umbilicus, liver —1½″ below the costal margin. (3) Ascites present.

Blood examination report on admission on 6-5-41 :Hb—45%, R. B. C.—2.2 mill., W. B. C.—3,125, Poly—56%, Lympho—40%, Mono—1%, Eosino—3%. M. P.—nil. Aldehyde test—strongly positive. Spleen puncture—L. D. bodies found

Treatment : Paracentesis abdominis was done twice and neostibene was given intramuscularly twice a week. Total dose given was 4 gms.

Results of treatment on 12-7-41 : (1) No fluid was present in peritoneal cavity. (2) Temperature came down to normal after 12 injections of 0·1 gm. (3) Spleen—2½″ and liver—½″ below the costal margin. (4) *Blood report on 12-7-41* : Hb—65%, R. B. C.—3·8 mill., W. B. C.—4,737, Poly—65%, Lympho—29%, Mono—2%, Eosino—4%. No L. D. bodies found on spleen puncture.

Case V

Sufal Sardar, Hindu male, æt. 40, was admitted into the Tropical ward of the Carmichael Medical College Hospital on 26-2-41 with the following complaints : (1) Fever (double rise of temperature)—2 years. (2) Spleen and liver extended 4″ and 2½″ respectively below the costal margin. (3) Œdema of lower extremities—6 months.

Blood examination report on admission on 26-2-41 : Hb—45%, R.B.C.—2·3 mill., W.B.C.—2,500, Poly—54%, Lympho—42%, Mono—2%, Eosino—2%. M.P.—nil. Aldehyde test—strongly positive. Spleen puncture—L. D. bodies found.

Treatment : Neostibene was given intramuscularly twice a week. Total dose injected was 3·5 gms.

Results of treatment on 6-4-41 : (1) Temperature came down to normal after 10 injections of 0·1 gm. (2) Liver not palpable, spleen 1″ below the costal margin. (3) Blood report on 6-4-41 : Hb—60%, R. B. C.—3·2 mill., W. B. C.—4,737, Poly—70%, Lympho—25%, Mono—2%, Eosino—3%. No L. D. bodies found on spleen puncture.

Case VI

Bhadro Kanto Jha, Hindu male, æt. 16, was admitted into the Tropical Ward of the Carmichael Medical College Hospital on 16-9-41 with the following complaints : (1) Irregular intermittent fever—6 months. (2) Spleen and liver extended 4″ and 2″ respectively below the costal margin.

Blood examination report on admission on 16-7-41 :
Hb—40%, R. B. C.—2·2 mill., W. B. C—3,125, Poly—
60%, Lympho—39% , Mono—nil, Eosino—1%. M. P.—nil.
Spleen puncture—L. D. bodies found. Aldehyde test—
positive.   •

Treatment : Neostibene 1 gm. has been injected intra-
muscularly and the patient is still under treatment.

Results of treatment on 5-8-41 : (1) Liver and spleen were
1′ and ′3″ respectively below the costal margin. (2) Blood
report: Hb—50%, R. B. C.—3 mill., W. B. C.—3,749,
Poly—66%, Lympho—33%, Mono—nil, Eosino—1%. No
L. D. bodies found on spleen puncture. (3) The patient
became afebrile after 6 injections of 0.1 gm.

Case VII

Hari Sadhan Maity, Hindu male, æt. 28, was admitted
into the Tropical Ward of the Carmichael Medical College
Hospital on 15-6-41 with the following complaints : (1) Ir-
regular intermittent fever—3 years. (2) Liver and spleen ex-
tended 3″ and 5″ respectively below the costal margin.
(3) Jaundice present.

Blood examination report on admission on 15-6-41 :
Hb—35%, R. B. C.—2 mill., W. B. C.—2,500, Poly—65%,
Lympho—30%, Mono—2%, Eosino—3%. M. P.—nil.
Spleen puncture—L. D. bodies found. Aldehyde test—
positive.

Treatment : 2 gms. of neostibene have been injected
intramuscularly twice a week. Iron and liver preparations
have been given also.

Results of treatment on 5-8-41 : Hb—50%, R. B. C.—
2·8 mill., W. B. C.—3,125, Poly—68%, Lympho—30%,
Mono—nil. Eosino—2%. Liver—1″ and spleen—3″ below
the costal margin. General condition much improved.

Case VIII

Makhan Lal Ghosh, Hindu male, æt. 28, was ad-
mitted into the Tropical Ward of the Carmichael Medical

College Hospital on 6-7-41 with the following complaints:
(1) Fever (double rise of temperature)—2 years. (2) Chronic
bronchitis present—6 months. (3) Spleen and liver extended
3″ and 1½″ respectively below the costal margin. (4) Epis-
taxis—2 months.

Blood examination report on admission on 6-7-41 : Hb—
40%, R. B. C.—1·9 mill., W B. C·—2,500, Poly—60%,
Lympho—38%, Mono—nil, Eosino—2%. M.P. nil. Spleen
puncture—L. D. bodies found. Aldehyde test—positive.

Treatment : Neostibene was injected intramuscularly twice
a week. Total dose given was 1·5 gm. during one month.

Results of treatment on 4-8-41 : (1) Liver—palpable,
spleen –1″ below the costal margin. (2) Blood report :
Hb—50%, R B. C.—2·5 mill., W. B. C –3,125, Poly—
65%, Lympho—30%, Mono—4%, Eosino—1%. The
patient became afebrile after 8 injections of 0·1 gm.

Case IX

Khudiram Saha, Hindu male, æt. 45, was admitted
into the Tropical Ward of the Carmichael Medical College
Hospital on 8-6-41 with the following complaints : (1) Irregu-
lar intermittent fever—3 years. (2) Liver and spleen extended
2′ and 4″ respectively below the costal margin. (3) Cough
with expectoration—6 months.

Blood examination report on admission on 8-6-41 : Hb—
45%, R. B. C.—2·2 mill., W. B C.—3,125, Poly—61%,
Lympho—38%, Mono—nil, Eosino—1%, M. P.—not found.
Spleen puncture L. D. bodies found. Aldehyde test—posi-
tive.

Treatment : Neostibene was given intramuscularly
twice a week. Total dose given was 1.5 gm. during a
month's time.

Results of treatment on 9-7-41 : (1) Hb—55%,
R. B. C –3 mill., W. B. C.—4,625, Poly—68%, Lympho—
28%, Mono—2%, Eosino—2%. (2) Liver—not palpable,

spleen—1″ below the costal margin. (3) The patient became afebrile after 9 injections of 0·1 gm. No L. D. bodies found on spleen puncture.

Case·X

Rajab Ali, Mahomedan male, æt. 32, was admitted into the Tropical Ward of the Carmichael Medical College Hospital on 20-3-41 with the following complaints : (1) Irregular intermittent fever—5 years. (2) Profuse epistaxis—1 month. (3) Spleen and liver extendded 5″ and 3″ respectively below the costal margin. (4) General weakness and emaciation—5 years.

Blood examination report on admission on 21-3-41. Hb 35%, R. B. C.—1.9 mill., W. B. C.—2,500, Poly—60%, Lympho—39%, Mono—nil, Eosino—1%. No M. P. found, Aldehyde test—weakly positive. Spleen puncture—L.D. bodies found.

Treatment :. Neostibene was injected intramuscularly twice a week. Total dose given was 1·5 gm Iron and liver preparations were also injected Calcium gluconate was given intramuscularly for one month.

Results. of treatment on 10-5-41 : (1) The patient became afebrile after 8 injections of 0·1 gm. (2) Spleen and liver—3″ and 1″ respectively below the costal margin. (3) Body weight increased by 10lbs (4) Blood report on 8-5-41 : Hb—50%, R. B. C.—2.8 mill., W.B.C.—3,125, Poly—68%, Lympho—30%, Mono - 1% Eosino—1%. No L.D. bodies found on spleen puncture. Further investigation is in progress.

I am deeply indebted to my house physicians for keeping careful records of the cases in my ward.

Editor's Note

A second paper on further series of cases treated with neostibene is under publication in the December issue of the Journal of Tropical Medicine and Hygiene.